3. Research Center for Charged Particle Therapy

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(Outline of Research Career)

Dr. Kamada received a Ph.D. from Hokkaido University in 1996 for his study on radiotherapy of bile duct cancer. He has had 29 years of experience in clinical research on radiation oncology, including 14 years experience in carbon ion radiotherapy at NIRS. Since 2006, he has been group leader of the diagnosis and treatment advancement research group for standardization and improvement of therapeutic and diagnostic techniques. He has been the Director of the Research Center for Charged Particle Therapy, NIRS since 2008.
Objectives
The Research Center for Charged Particle Therapy (hereafter, abbreviated as "the Center") was established in 1993 when the NIRS completed construction of the HIMAC. Since then it has been carrying out clinical, biological and physics research using heavy ions generated from the HIMAC. After accumulating clinical experiences with carbon ion radiotherapy in various types of malignant tumors, in 2003 the Center was successful in obtaining approval from the Ministry of Health, Welfare and Labor for "Highly Advanced Medical Technology".
Carbon ion therapy has in the meantime achieved for itself a solid place in general practice of cancer treatment. The HIMAC has also served more than 500 researchers as a multi-user utilization facility for medical, biological and physics research.
In 2006, when the second mid-term plan of the NIRS was initiated, the Center was reorganized to conduct life science research on ionizing radiation, focusing on carbon ion radiotherapy. This would eventually contribute to the improvement of the quality of life of human beings. Research plans for FY 2008 included: clinical study on carbon ion radiotherapy for locally advanced tumors; development and improvement of radiotherapeutic techniques; design study and R&D for a new extension of the treatment rooms for the HIMAC; research on diagnostic imaging; QA/QC for radiotherapy and radiation protection; radiobiological experiments for improvement of radiotherapy; exploration of variability of radiation sensitivity by investigating SNIPs; and research on HiCEP.

Overview
The Center is organized as six research groups for two major topics (A and B) and one invited research project (C). Research progress for each topic is summarized below.
A. Research on the use of heavy ion beams for cancer radiotherapy
   ① Development of advanced cancer radiotherapy with charged particles
   This research subject has been pursued by the Particle Therapy Research Group (GL: H. Tsuji) using three teams: Clinical Trial Research Team, Clinical Database Research Team, and Radiation Effect Research Team. The Clinical Trial Research Team has continuously increased the number of patients treated each year; in FY 2008, 684 patients, the maximum number ever, underwent carbon ion radiotherapy (C-ion RT). So far, a total of 4504 patients have been enrolled in clinical trials of C-ion RT and prostate, lung, head and neck, bone and soft tissue, and liver tumors were the leading five tumor types in the trials. The outcomes of the clinical trials revealed that the C-ion RT provided definite local control and offered a survival advantage without unacceptable morbidity in a variety of tumors that were hard to cure with other modalities. In addition, it was possible to implement hypofractionated radiotherapy by using carbon ion beams, with application of larger doses per fraction and a reduction of overall treatment times as compared to conventional photon radiotherapy. In particular, clinical trials of ultra-short course C-ion RT for lung cancer (single fraction) and liver cancer (two fractions) have been successfully achieved. Additionally, advancement of hypofractionation has also been made in other tumor entities. For instance, the fraction number in the treatment of prostate cancer could successfully decrease from 20 to 16, with even lower incidence of late toxicity and comparable outcomes in tumor control.
   Developments in the technology of the beam delivery system, a new multi-leaf collimator (MLC) and a new method for manufacturing range compensators have also been carried out for the sake of improving treatment efficiency. The range compensator fabricated by a new method was actually used in the treatment of prostate cancer patients this year. The Clinical Database Research Team has achieved improvement of the coordination among several database systems (Hospital Information System, Therapy Plan Database, Therapy Schedule Management System, two PACS and the Radiology Information System for Radiation Therapy). The developed information systems conforming to the functions, Integrating the Healthcare Enterprise (IHE)-Enterprise User Authentication (EUA) and Patient Synchronized Applications (PSA), made it easy to operate multiple systems in one clinical unit. As a result, the developed system contributed to the improvement of efficiency of patient registration and a resultant increase in the number of patients. In addition, the functions for analyzing the data of the database system were improved and basic analysis, such as the Kaplan-Meier estimate of patient survival, became much easier than before. The Radiation Effect Research Team has aggressively performed experiments and analysis as well. The radiosensitivity analysis based on the TCP model was applied to the analysis of late toxicity on the genitourinary (GU) system in prostate cancer. The results revealed that the $\alpha/\beta$ value of the GU was substantially larger than that for photons in literature but the BED calculated with the $\alpha/\beta$ value for the carbon ion beam was consistent with that for photons. The skin reaction of mice was investigated by fractionated irradiation experiments with carbon beams. As a result, it was found that the effect of single fraction irradiation differs uniquely from those by multiple fractionations: the efficacy tends to be small for single fractions. Lineal energy information measured by the tissue-equivalent proportional counter in the therapeutic irradiation field was obtained to estimate biological effectiveness of the beam. The effect of the
field size in the small field treatment and the difference in the biological dose distribution due to the shape of the ridge filter were investigated in FY 2008. It was found the detected effect and difference did not have any serious influence on the current clinical application of carbon ion beams.

Development of a novel irradiation system for charged particle therapy

This research subject has been pursued by the Medical Physics Research Group (GL: K. Noda) using four teams: Accelerator Development Research Team, Irradiation System Research Team, Therapy System Research Team, and Compact Heavy Ion Therapy System Research Team.

Based on more than ten years of experience with HIMAC, the group has proposed a new treatment facility moving toward adaptive cancer therapy with heavy ions, which makes the one-day treatment of lung cancer possible. In the new treatment facility, it should be possible to accurately treat a fixed target, a moving target with breathing and/or a target near a critical organ.

For these purposes, a 3D-scanning method with a pencil beam is employed in the new treatment facility. A phase-controlled rescanning (PCR) method has been proposed, especially for treating a moving target. In the PCR method, the fast 3D raster-scanning is one of the essential key technologies needed to irradiate a tumor within a tolerable time even scanning several times in each slice. For the fast 3D scanning, we have developed the following technologies: 1) a new treatment planning; 2) an extended flat-top operation of the synchrotron; and 3) a fast-scanning magnet system. In order to verify this method, we have designed and constructed a test irradiation port with which we carried out an experiment on the fast 3D raster-scanning. From preliminary findings, we verified that the irradiation time was significantly reduced compared with conventional spot scanning. In the new treatment facility, a rotating gantry with the PCR method will also be employed in order to reduce the treatment burden on the patient and to increase the treatment accuracy for tumors near a critical organ when the multi-field optimization method is used. After the beam-optics design, a mechanical design was carried out. As a result, the weight of the gantry is held to 350 tons, which is about half of that of the HIT gantry. Furthermore, for the multi-field optimization, inverse-planning has been studied. We verified that the method can reduce the dose in the OAR significantly while keeping it in the target. Including the studies mentioned above, for the new treatment facility, since April 2006, we designed a fixed beam delivery system, a rotating gantry system, a treatment management system, a patient-positioning system and a treatment planning system. The new treatment facility is connected with the existing HIMAC accelerator complex and the heavy ion beams are delivered to three treatment rooms. Two of them are equipped with both horizontal and vertical fixed beam delivery systems, and the other has the rotating gantry. Construction of the building for the new treatment facility was started in February 2009.

Standardization and improvement of therapeutic and diagnostic techniques

This topic covers a wide range of research which has been carried out by the Diagnosis and Treatment Advancement Research Group (GL: T. Kamada) with four teams: Image Diagnosis Research Team, Image Processing Research Team, Quality Control Research Team, and Radiological Protection Research Team.

The Image Diagnosis Research Team studied two PET tracers, ¹¹⁸⁷Cu-ATSM and C-11-methionine (MET), for oncologic imaging. This fiscal year, tumor hypoxic imaging using ¹¹⁸⁷Cu-ATSM was continued and primary brain tumor imaging using C-11-MET was also investigated. The team assessed whether Cu-62-ATSM imaging of tumor hypoxia is associated with C-11-MET imaging of amino acid metabolism in 18 patients with cervical cancer, and found that tracers showed different distribution patterns in same patient. For brain tumor imaging using C-11-MET, we found that its accumulation was well correlated to the histopathologic grade of glioma.

In FY 2008, the Image Processing Research Team analyzed intrafractional organ movement during respiration using 4D CT (256MSCT) in patients with lung carcinoma. Interfractional volumetric cine imaging of the lung using 4D CT showed continuous movement of the tumor in the sagittal section satisfactorily. The 256MSCT significantly improved the observation of tumor displacement and overcame some of the limitations of present CT methods in lung cancer treatment.

The Quality Control Research Team carried out studies with regard to dosimetry for the therapeutic hadron beam. The team conducted a nation-wide proton dosimetry intercomparison that involved new proton facilities in Japan. The intercomparison results showed facilities had good agreement within 0.4% and dose uniformity was established among them.

The Radiological Protection Research Team measured organ doses of patients in CT screening using an adult anthropomorphic phantom and TLDs under CT scan conditions routinely used at two hospitals. The estimated equivalent doses of thyroid, lung, esophagus, breast, liver and stomach were between 0.8 and 2.6mSv. Based on evaluation of image quality for the CT images as well as estimated doses, adequate CT scan conditions were suggested considering the optimization of radiation protection in CT screenings.
for lung cancer.

B. Research on radiation effects for improvement of radiation therapy

1. RadGenomics research concerning radiation sensitivity

This research subject has been pursued by the RadGenomics Research Group (GL: T. Imai) using three teams: Genetic Information Team, Molecular Radiooncology Team, and Molecular Biostatistics Team.

Normal tissue reactions of cancer patients vary considerably after radiotherapy. A number of observations have indicated that certain genetic factors play important roles in this variability. The aim of the RadGenomics Research Group is to explore the genetic characteristics for both the patient and the bearing tumor, by which the potentially most effective radiotherapy can be delivered. From a molecular biological standpoint, this would open the way to the development of an individual-oriented radiotherapy.

This research group has focused on searching genetic predictive markers for clinical radiosensitivity of normal tissues and tumors. The clinical radiosensitivity of normal tissue is likely to be a complex trait that is dependent on the cumulative effect of many minor genetic determinants. We have searched for polymorphisms associated with the radiosensitivity of normal tissue in cancer patients who have undergone radiotherapy. Between October 2001 and March 2009, 2,653 patients were recruited for our project, including 773 breast cancer patients and 855 prostate cancer patients. The candidate genes for SNP typing in this project were selected from our previous comprehensive gene expression analyses data using human cultured cell lines and mouse strains. A total of 190 genes were chosen and 1,300 SNPs have been typed using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system.

In FY 2008, we indentified multiple SNPs associated with risk of urinary morbidity after carbon ion radiation therapy in prostate cancer patients. The data suggest that patients with late urinary morbidity after carbon ion radiotherapy can be stratified according to the total number of risk genotypes they harbor.

To obtain haplotype information for individuals, we developed a new analysis method for amplification of long DNA fragments. A limited amount of cellular DNA was released from intact cells into a mildly heated alkaline agarose solution and mixed. The solution was then gently aliquoted and allowed to solidify while maintaining the integrity of the diluted DNA. Exogenously provided Phi29 DNA polymerase was used to perform consistent genomic amplification with random hexameric oligonucleotides within the agarose gels. Simple heat melting of the gel allowed recovery of the amplified materials in a solution of the polymerase chain reaction (PCR)-ready form. The haplotypes of seven SNPs spanning 240 kb of the DNA surrounding the human ATM gene region on chromosome 11 were determined for 10 individuals. Our technique will facilitate determination of individual haplotypes and enhance predictive power for individual radiation sensitivity.

Recently, radiotherapy has been applied to many more patients as one of the best clinical modalities. To layer several kinds of treatments, it is necessary to know the effectiveness of radiotherapy even during it. While the pre-treatment status of cancer is generally correlated with outcome, little is known about micro-environmental changes caused by anti-cancer treatment and how they may affect outcome. We attempted to find a gene that was both induced by irradiation and associated with radiosensitivity in tumors. We analyzed the gene expression profiles of two murine carcinomas, NR-S1, which is highly radiosensitive, and SCCVII, which is radiosensitive, after irradiation with gamma rays or carbon ions. Four genes, Efnal (Ephrin-A1), Sprtla, Srgap3 and Xtra1, were selected as candidate genes associated with all kinds of radiotherapy-induced radiosensitivity. We focused on Efnal, which encodes a ligand for the Eph receptor tyrosine kinase that is known to be involved in the vascular endothelial growth factor (VEGF) pathway. Ephrin-A1 was detected in the cytoplasm of NR-S1 tumor cells after irradiation, but not in SCCVII tumor cells. Irradiation of NRS1 tumor cells also led to significant increases in microvascular density and up-regulation of VEGF expression. Our results suggest that radiotherapy-induced changes in gene expression related to angiogenesis might also modulate micro-environments and influence responsiveness of tumors.

2. Biological research concerning the improvement of radiation therapy

This research subject has been followed by the Heavy Ion Radiobiology Research Group (GL: R. Okayasu) with four teams: Biophysics Team, Experimental Therapy Team, Cellular and Molecular Biology Team, and Radiation Modifier Team.

The Biophysics Research Team proposed an experimental fitting function of the LET-RBE relationship to estimate RBE for unknown ion beams at a defined LET. was Experimental LET-RBE spectra of cell survival for different ion beams were fitted by the LQ-model to obtain parameters for the function . The spectra were analyzed with the function, and clear splits of the spectra were found among various ion species. Those parameters can be defined as functions of atomic numbers of ion beams. This method is applicable to estimate overall RBEs in the therapeutic beams because each beam must consist of different ions having different RBEs when passing through a patient body or other absorbing materials in order to adjust the
beam range in the body.

RBE values for 20% tumor induction in mice by carbon ions at LET of 15, 45, 75 keV μm are 0.6, 1.0 and 1.4 respectively. To determine variations in the sensitivity of tumors having mixed populations with different sensitivities, the Experimental Radiotherapy Research Team has carried out experiments on mice with tumor cells with resistant and sensitive populations. When more than 10% of the cells were resistant, the overall sensitivity was very similar to that when 100% of the cells were resistant. To investigate the relationship between LET and skin reaction, the team performed fractionated irradiation on the normal mouse foot. The a/β ratios were 28 and 39 Gy for LET values of 58 and 14 keV μm, respectively, and 38 Gy for γ rays. There seemed to be no significant differences in the a/β ratios for different LETs.

The Cellular Molecular Biology Research Team used the comprehensive gene expression technique (HiCEP) with irradiated human cell lines to demonstrate some characteristic molecular signatures for different types of ionizing radiation (IR) at therapeutic doses. A group of early responsive IR-induced genes (ATF3, BTG2, TFR1) in human cells were found to remain active for a longer period with carbon ions than with X-rays. In addition, we successfully detected some common genes which were down-regulated by various types of IR. We also demonstrated that cells irradiated with X-rays and heavy ion particles showed different radiosensitivities depending on the DNA repair characteristics of the cells; in particular, homologous recombination (HRR) defective cells showed an extremely high sensitivity to high LET heavy ion irradiation.

To develop a better free radical scavenger, the Radiation Modifier Research Team performed a kinetic study on the free radical scavenging reaction of vitamin E precursors. The electron-donating groups on the benzene ring of p-hydroquinones significantly enhanced the scavenging activity, based on the second-order rate constants determined by the stopped-flow technique. From the study of in vivo radiation-protectors and -mitigators, it was found that several compounds effective against low LET radiation-induced injury were also effective to protect against bone marrow death or to mitigate the number of bone marrow deaths in mice induced by carbon ion irradiation. A new data acquisition method for CW-ESR spectral-spatial imaging was proposed. Details of reactions between a nitroxyl probe and glutathione were investigated, and the depth-dependent free radical generation by carbon ion irradiation in gelatin sample containing the nitroxyl probe and glutathione was detected using ESR and MRI methods.

This subject has been followed by the Transcriptome Research Group (GL: Abe) which consists of three teams: Stem Cell Research Team, Gene Expression Profiling Team, and Model Organism Research Team.

To fully understand genetic information residing in the entire genome is the next major goal in life science and also an important issue for radiobiology. However, the actual number of transcripts expressed from the whole genome is quite large and still unknown, more than 40,000 per cell or more than 150,000 per individual. Thus far, no way has been available to detect such large numbers of transcripts. The transcriptome Research Group has developed a new method for gene expression profiling called High-Coverage gene Expression Profiling (HiCEP). HiCEP enables detection of 30,000 ≤ 40,000 transcripts per cell, allows observation of expression differences of as little as 1.2 times, and allows detection of unknown, very slightly expressed genes. Because this method does not require any sequence information in advance, it can be used for all species, rather than only for the usual laboratory animals. This is another great advantage of this method.

So far, we have successfully observed changes in gene expression of two-fold or less with good reproducibility after irradiation, identified novel rhythmic expressed transcripts in the suprachiasmatic nucleus, which is a minute nucleus in the brain and governs the biological clock, and identified many genes including unknown ones whose expression was deregulated only in gene knockout mouse. All of these would be difficult for conventional methods to detect and analyze.

Issues to be addressed for further successful implementations of HiCEP included the development of: 1) techniques for the mass processing of samples (Fig.3-1); 2) analytical techniques for small amounts of samples; 3) a kit that enables anyone to perform the HiCEP reaction; 4) a rapid peak isolation system after the analysis; and 5) a mass information processing system. Our current progress on each issue is summarized as follows. 1) An auto-machine named HiCEPer was developed that can carry out the HiCEP reaction for 96 samples simultaneously. 2) Reaction using only 100 cells became possible and we are attempting to develop a reaction system using only 10 cells. 3) A kit using 1 microgram total RNA is now available. 4) A new technology using a microchip was developed and we are constructing a test machine in which the microchip technology was included. 5) Several useful software codes have been developed for HiCEP analysis.

Progress in the construction of these systems has been and is being made by the Development of Systems and Technology and Analysis Project of the Japan Science and Technology Agency and NIRS.
These R&D projects are necessary to encourage the dissemination of this new method over various scientific fields including basic sciences, molecular epidemiology and clinical medicine.

C. Research Project with Heavy Ions at NIRS-HIMAC

In FY 2008, 141 proposals were accepted and carried out at HIMAC. The beam time of 5549 hours was supplied to those studies. Ninety-eight papers and 29 proceedings were published, and 299 papers were presented at various meetings. A total of 530 researchers participated, including 44 foreign researchers for 15 international projects.
3.1 Developing Advanced Clinical Therapy with Charged Particles

Hiroshi Tsuji, MD, Ph.D.
Director, Particle Therapy Research Group

(Outline of Research Career)
Dr. Tsuji received a Ph.D. from Tsukuba University in 1996 for his study on proton radiotherapy of hepatocellular carcinoma. He has had 26 years of experience in clinical research on radiation oncology, including 14 years experience in carbon ion radiotherapy at NIRS. Since 2008, he has been group leader of the Particle Therapy Research Group for developing advanced clinical therapy with charged particles.
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Objectives

- Carry out clinical studies to develop therapeutic techniques for diseases that are difficult to treat with other therapies (such as pancreatic cancer) and for which charged particle radiation therapy does not yet have a role.
- Carry out studies on optimizing irradiation methods by disease and by region, using clinical investigations of therapies in which radiation is combined with drugs and operations.
- Develop a comprehensive database on treatment, clinical course and other factors. Compare and analyze domestic and foreign data on particle beam therapy.
- Provide annual treatment to 500 patients to maximize and disseminate the therapeutic effects of charged particle technology. This is the target number combining patients taking part in clinical studies and those receiving high-technology treatments, and is based on consideration of the fact that the NIRS is primarily a research and development facility.
- Evaluate the therapeutic effects of treatments developed by NIRS from the viewpoints of quality of life (QOL) and therapeutic costs. Patients' opinions are collected to gauge their level of satisfaction with the therapy.

Progress of Research

The Particle Therapy Research Group for developing advanced clinical therapy with charged particles consists of the Clinical Trial Research Team, Clinical Database Research Team, and Radiation Effect Research Team. It does research and development on charged particle therapy. Progress of research in each team is summarized below.

1) Clinical Trial Research Team

From June 1994 to February 2009, a total of 4504 patients were enrolled in clinical trials using carbon ion beams generated by HIMAC. Carbon ion radiotherapy of these patients was carried out by nearly 50 different phase I/II or phase II protocols and highly advanced medical technology. Figure 3-1 lists the number of the patients for each tumor site treated with carbon ion beams.

Fig.3-1. The number of patients for each tumor site treated with carbon ion beams.

We treated 684 new patients in FY 2008. Prostate, lung, head and neck, bone and soft tissue, and liver tumors are the leading five tumor types in the trials. A total of 3820 patients who had a follow-up period of 6 months or more were included in this report. The clinical trial revealed that carbon ion radiotherapy provided definite local control and offered a survival advantage without unacceptable morbidity in a variety of tumors that were hard to cure with other modalities. Using carbon ion beams, it was possible to implement hypofractionated radiotherapy, with application of larger doses per fraction and a reduction of overall treatment times as compared to conventional photon radiotherapy. Carbon ion radiotherapy has been approved by the Ministry of Health, Labor and Welfare of Japan as “Highly Advanced Medical Technology (HAMT)” since November 2003. In 2008, nearly 75% of the patients receiving carbon ion radiotherapy were treated by HAMT.

When irradiating a patient with carbon beams, the patient should be protected from exposure to an unwanted dose. A multi-leaf collimator (MLC) and patient collimators are used to spatially limit the carbon beams for the sake of delivering high localization of the dose to a target. The MLC can easily form an arbitral aperture shape by computer control. However, since each leaf is 6.5 mm thick, it is difficult to make the fine shape which is required for the cases of cancers which are abutting critical organs. It these cases, a patient collimator is used, which is manufactured by boring an aperture in a brass block; this takes a few days and is costly. Furthermore, use of the patient collimator has required radiation therapy technologists set the heavy collimator just above a patient in positioning. Omitting use of the patient collimator reduces the expense and the human burden.

A new MLC has been developed which is equipped with 88 pairs of a 2.5 mm thick leaf with 0.15 mm spacing. This thickness is almost 1/3 of the present thickness of 6.5 mm. We experimentally proved that the leakage dose of the MLC was about 1% of the unshielded dose compared with the 0.6% leakage dose of the present MLC. Of particular interest in the study...
was identifying what particles contribute to the leakage dose. Protons were experimentally proven to be the biggest contributor and helium ions, the next biggest. Heavier particles, except for carbon, contribute only slightly to the dose.

Range compensators are also essential in the broad beam method. At present, polyethylene blocks are machined by a numerically controlled device to manufacture the range compensators, a time-consuming process that can take as long as eight hours. Then, cleaning and inspection will take about another hour. We developed a new method for manufacturing range compensators, employing a punch technology. The compensator is assembled by lamination. Each plate is 3 mm thick, the distal end shape is punched out from the plate, and then the shape is inspected automatically. The plates are stacked up at the end stage of the process. The laminated block is manually tightened with bolts. This simple process has greatly shortened the manufacturing time, as punching and stacking take half an hour or less.

2) Clinical Database Research Team

In October 2006, we implemented the Electronic Medical Record (EMR) system and developed a simple input method for each patient’s findings which include symptoms, tumor responses, and toxic reactions that should be estimated by the physician during the clinical interview. We improved the coordination among several database systems (Hospital Information System, Therapy Plan Database, Therapy Schedule Management System, two PACSs and Radiology Information System for Radiation Therapy). These systems are connected to each other and data are transmitted to the designated systems.

We also developed information systems that conform to the functions of Integrating the Healthcare Enterprise (IHE)-Enterprise User Authentication (EUA) and Patient Synchronized Applications (PSA). These functions make it easy to operate multiple systems. Two PCs (one for the EMR and one for the PACS viewer) are commonly used for the Hospital Information System in one clinical unit. Many physicians have to enter a user ID and password to log into these systems. The developed functions of the IHE-EUA and PSA ease this troublesome manipulation. We developed middle-ware for the EUA and PSA functions to reduce the implementation load among the EMR, PACS-viewer, report-viewer, radiation scheduling system, and radiation information system. We realized that EUA and PSA functions were essential in a multi-system environment. Our middle-ware resolved the complexities of the application implementation. The established guideline was useful to unify the user interfaces of each application. We found that the EUA and PSA functions are critical for visual integration.

We implemented a system to share medical data between hospitals and medical institutions. This system is based upon the IHE Cross-Enterprise Document Sharing (XDS) which uses SOAP, ebXML RIM and Web Service Description Language (WSDL) and HL7. We prepared the Open Source Software license for the delivery of software. We are now developing the document source, document repository, document registry and document consumer that were defined by the IHE XDS. We had previously developed the application software. We are now modifying them according to the newer IHE version. We think that it is very important to maintain this software and to improve the code periodically. We are working to establish a maintenance framework for the open source software.

We have a clinical database system which contains information concerning over 4,000 patients who have had heavy particle radiation therapy and over 18,000 patients who have had photon radiation therapy. We improved this database system in its processing speed and ease of operation. By using this system physicians can analyze patients by the heavy particle radiation therapy protocol and generate survival curves in a few seconds. This database can store data concerned with the disease history, staging, radiation schedule, radiation dose/days, adverse effects and follow-up information.

The NIRS Hospital Information System was modified in 2008 and its status in January 2009 is shown in Fig.3-2.

Fig.3-2. Current status of the NIRS Hospital Information System.

3) Radiation Effect Research Team

The radiosensitivity analysis based on the TCP model has been applied for the analysis of toxicity on benign tissue. Late toxicity on the genitourinary (GU) tract observed during treatment of prostate cancer with carbon ions was analyzed with the model. The analysis revealed that the ab value of the GU was 7.7, which was more than 2 times larger than that for photons (3.0) in the literature. BED calculated with the $a/β$ value for the carbon ion beam was 73.8, which was consistent with that for photons, 74.7. This information
will contribute to the prospective estimation of prescribed dose in different fractionations or to further dose optimization in treatment planning.

Reaction of the skin is one of the most important endpoints to be regarded in radiotherapy; however, its analysis from clinical outcomes is not easy as radiation quality and the dose given to patients significantly differ among individuals. From this viewpoint, skin reaction has been investigated through reaction observations on mice. Through the fractionated irradiation of carbon beams to mouse leg, we found that the effect of single fraction irradiation differs uniquely from those by multiple fractionations: the efficacy tends to be small on single fractions.

Linear energy information measured by a tissue-equivalent proportional counter in the therapeutic irradiation field is found useful for the sake of estimating biological effectiveness of the beam at the point by processing the information with the Microdosimetric Kinetic Model (MKM). In FY 2008, the method was applied for the verification of actual irradiation fields and the following results have been obtained.

Field effect
In the case of a small irradiation field, the decrease in the absorbed dose at the center of the irradiation field by collimator is almost completely compensated by the increase in radiation quality. The resultant isoeffective dose is regarded as stable.

Port characteristics
Due to the machining precision of ridge filters, therapeutic beam distribution could differ port-by-port. Verification of the port dependency by the MKM revealed a slight difference in radiation quality though that in the absorbed dose was negligible. However, the absolute difference in the isoeffective dose was small and it was confirmed that the therapeutic beam provided in each port can be regarded as identical.

Major publications
5) T. Sugane: Carbon ion radiotherapy for elderly patients 80 years and older with stage I non-small cell lung cancer. Lung Cancer, 64[1], 45-50, 2009
6) T. Yanagi: Mucosal malignant melanoma of the head and neck treated by carbon ion radiotherapy, International Journal of Radiation Oncology Biology Physics, 74[1], 15-20, 2009
3.2 Research on the Next-generation Irradiation System

Koji Noda, Ph.D.
Director, Medical Physics Research Group

(Outline of Research Career)

Dr. Noda received his B.S. degree from the Department of Nuclear Engineering, Kyushu University in 1979. After completing the M.S. program there in 1981, he worked on development of a PET cyclotron from 1981 to 1989. He also studied accelerator physics from 1985 to 1989 in the Institute for Nuclear Study, University of Tokyo. In 1989, he joined the HIMAC project at NIRS and he was engaged in construction and development of the HIMAC synchrotron. He received his Ph.D. in 1992 from Kyushu University for the study of energy-loss cooling. Currently he is head of the Accelerator Development Section and he holds the additional post of Director of the Medical Physics Research Group.

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Objectives

Based on more than ten years of experience with HIMAC, we have proposed a new treatment facility leading toward adaptive cancer therapy with heavy ions which makes the one-day treatment of lung cancer possible. Further, the new treatment facility should be able to accurately treat a fixed target, a target moving because of breathing, and/or a target near a critical organ. For these purposes, a 3D-scanning method with a pencil beam will be employed in the new treatment facility. A phase-controlled rescanning (PCR) method has been proposed and studied, especially for treating a moving target. A rotating gantry with the PCR method is also employed in order to reduce the burden on the patient, and to increase the treatment accuracy for a tumor near a critical organ through the multi-field optimization method. Therefore, we have designed a fixed beam delivery system, a rotating gantry system, a treatment management system, a patient positioning system and a treatment planning system, and the related R&D work has also been carried out with HIMAC since April 2006. Construction of the building for the new treatment facility was started in February 2009.

Progress of Research

1) Planning of the new treatment facility

The new treatment facility, as shown in Fig. 3-3, is connected to the existing HIMAC accelerator complex and heavy ion beams are delivered to patients through the fixed irradiation port and the rotating gantry. In the treatment hall, placed underground in the facility, there are three treatment rooms to allow treatment of around 1000 patients per year. Two of them are equipped with both horizontal and vertical fixed beam delivery systems, and the other is equipped with the rotating gantry. The 3D raster-scanning method is employed in both the fixed beam delivery and rotating gantry systems. In order to carry out treatment of a moving target as well as a fixed target, the PCR method, which completes the irradiation on one slice during one respiration-gate opening, has been proposed and verified through computer simulation. The scanning speed should be faster than the conventional scanning method in order to complete treatment within a tolerable time, because rescanning naturally takes a longer time. Therefore we have developed fast 3D raster-rescanning with gating.

In cooperation with medical staff in the HIMAC hospital, the treatment hall planning has been carried out. Two treatment-simulation rooms are also prepared for patient positioning as a rehearsal, and for observing any change of the target size and shape during the whole treatment period with X-ray CT. Furthermore, six rooms are devoted to patient preparation before irradiation. The facility building construction began in February 2009.

The specifications of the facility are summarized at Table 3-1.

Table 3.11. Specifications of the new treatment facility

1. Basic parameters
   - Ion species: $^{12}$C, $^{16}$O ($^6$C, $^{12}$O)
   - Delivery beam intensity: $10^4$ — $10^5$ pps at $^{12}$C
   - Treatment room: 2 fixed beam rooms (Hori. & Vert.), 1 rotating gantry room

2. Fixed beam delivery system
   - Energy: 140 — 430 MeV/n
   - Irradiation method: Fixed target: 3D raster-scanning with pencil beam
   - Moving target: PCR method
   - Scanning speed: H: 100mm/ms, V: 50mm/ms
   - Spot size: 2—4mm at 1-sigma
   - Lateral field/ROBP/Range size: 22cm×22cm/15cm>25cm at $^{12}$C
   - Irradiation port length: 9m

3. Rotating gantry system
   - Type: Iso-centric rotating gantry
   - Energy: 140 — 400 MeV/n
   - Irradiation method: Same as the fixed beam delivery system
   - Scanning speed: H: 100mm/ms, V: 50mm/ms
   - Spot size: 2—4mm at 1-sigma
   - Lateral field/ROBP/Range size: 15cm×15cm/15cm>25cm at $^{12}$C
   - Displacement of iso-center: < 1 mm
   - Size and weight: Length: 16.5m, Radius: 7.1m, Weight: 350 tons

Fig.3-3. Schematic view of the HIMAC and the new treatment facility.

2) Related R&D work
   a) Development of accelerator technology
   b) In the present operation of the synchrotron, one cycle, consisting of a beam injection, acceleration and extraction, is made every 3.3 s. Within the cycle, the
beam will be extracted and irradiated onto a patient during approximately 2 s on the flattop of the synchrotron pattern. For moving targets, a respiration-gated irradiation was developed and it will also be used for the raster-scanning irradiation. For the present operation of the synchrotron, an inevitable dead-time exists in the respiration-gated irradiation, because the synchrotron requires a certain time for injection and acceleration, and the cycle of the synchrotron is fixed. This dead-time would make the total irradiation time longer. To overcome this problem, we developed the extended flattop operation. In this scheme, the beam is extracted by using the RF-KO extraction while the respiration gate is opened; this operation will significantly decrease the dead-time of the irradiation. This operation will also be applied to the raster-scanning irradiation in the new facility. The raster-scanning irradiation enables us to irradiate almost 100% of the beam particles on the target. Since the synchrotron ring of the HIMAC can accelerate a few tens of billions of carbon ions within one synchrotron cycle, and the numbers of carbon ions required to treat typical tumor sizes are on the order of 109 particles, most treatments can be completed within a single synchrotron cycle, provided that most of the accelerated particles are actually utilized in the treatment dose. Consequently, having applied the extended flattop operation to the raster-scanning irradiation, the total irradiation time is considerably to be decreased to a few seconds. This operation was successfully tested and implemented in the HIMAC accelerator control.

b) Experiment on fast 3D raster-scanning

We carried out the 3D raster-scanning experiment using the improved spot-scanning system in the secondary beam line. In this experiment, we verified fundamental performances of the dose distribution by the 3D raster-scanning, the PCR method and the treatment planning. As the next step, a test irradiation port was designed and installed (Fig. 3-4) in the HIMAC physics experimental line in order to verify experimentally the fast 3D raster-scanning and the PCR method. This test port has the same configuration as the fixed beam delivery system for the new treatment facility as shown in Table 3-1 and in Fig. 3-3. The scanning experiment has been on-going since December 2008. In the experiment, the extended flattop operation has been routinely utilized. A preliminary result verified that the scanning speed achieved the desired value.

Fig. 3-4. Test port for the fast 3D raster-scanning.

Major publications


3.3. Standardization and improvement of therapeutic and diagnostic techniques

Tadashi Kamada, MD, Ph.D.
Director, Diagnosis and Treatment Advancement Research Group

[Outline of Research Career]

Dr. Kamada received a Ph.D. from Hokkaido University in 1996 for his study on radiotherapy of bile duct cancer. He has had 28 years of experience in clinical research on radiation oncology, including 14 years of experience in carbon ion radiotherapy at NIRS. Since 2006, he has been group leader of the diagnosis and treatment advancement research group for standardization and improvement of therapeutic and diagnostic techniques. He has been a Director of the Research Center for Charged Particle Therapy, NIRS since 2008.

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Objectives

- Development of software to create integrated clinical images, determine early therapeutic effects and analyze prognostic factors using a combination of multiple diagnostic imaging techniques
- Improvement of treatment plans by using integrated images obtained from advanced dynamic imaging devices such as 4-dimensional CT
- Promotion of R&D on indicators of quality standards and methods for quality control and assurance of particle beam and photon beam therapies and of diagnosis using radiation
- Advancement and standardization of therapeutic and diagnostic methods based on investigation of medical radiation exposure in Japan.

Progress of Research

The Diagnosis and Treatment Advancement Research Group for standardization and improvement of therapeutic and diagnostic techniques consists of the Image Diagnosis Research Team, Image Processing Research Team, Quality Control Research Team and Radiological Protection Research Team. The group researched the advancement and standardization of radiation therapy and diagnostic methods. Progress of research in each team is summarized below.

1) Image Diagnosis Research Team

We studied fundamentals of application of new PET tracers for clinical diagnosis. The main targets of our interests were imaging of cell/tissue metabolic indicators leading to treatment effects especially of carbon ion radiotherapy (CIRT).

We continued to assess whether determining Cu-62 labeled diacetyl-bis (N (4)-methylthiosemicarbazone): (Cu-62-ATSM) imaging of tumor hypoxia is associated with C-11-methionine (MET) imaging of amino acid metabolism in cerebral cancer. PET/CT was performed in 18 patients with cervical cancer before CIRT for evaluation of both tumor hypoxia using Cu-62-ATSM and amino acid metabolism using MET. Fifteen patients also received both PET studies after CIRT. Data are being surely and steadily accumulated. We also started an assessment using Cu-62-ATSM PET for pancreatic cancer patients.

F-18-FLT PET imaging for head and neck cancers to assess the CIRT effect was continued in cooperation with the Diagnostic Imaging Group of the Molecular Imaging Center.

The role of C-11-MET PET for non-invasive grading between oligodendrogial tumor and other brain tumors was studied. Several investigations have shown that the prognosis of oligodendrogial tumor is dependent on their histological grade. C-11-MET PET imaging is one of the most sensitive techniques for visualizing primary brain tumors. Then we aimed at evaluating the relationship between the uptake of MET and histopathologic grading based especially on oligodendrogial tumor versus other brain tumors. We determined cerebral uptake of MET in 30 patients with histologically proven gliomas (22 male patients and 9 female patients: mean age, 46.9y; range, 14-75y). Grades I, II, III and IV lesions (based on the WHO grading class) numbered 3, 10, 9, and 8 lesions, respectively. There were 3 oligodendroglioma (grade II) and 1 anaplastic oligodendroglioma (grade III) in our cases. Ecat Exact HR+ PET scanner and Biograph DUO PET/CT were used for imaging in this study. A semi-quantitative MET uptake ratio (TNR; Tumor to normal tissue ratio) was correlated with tumor grade. We found there was a significant difference in TNR between grades III and IV lesions, but no significant difference between grades II and III in all the cases. In oligodendrogial tumors, 3 of the grade II lesions tended to show higher TNR than one of the grade III lesions. There was a significant difference in TNR between grade II and III lesions except oligodendrogial tumors from the cases. Oligodendroglioma might represent different metabolic demand for MET uptake from the other gliomas. We concluded that MET PET was sensitive for histopathologic grading of gliomas except oligodendrogial tumors. We must pay much attention to primary staging of oligodendrogial tumors using this tool.

Results were obtained from a study of C-11-MET PET imaging of choroidal melanoma and the time course after CIRT. In this work the team assessed the feasibility of MET-PET as an evaluation method of the therapeutic effect of CIRT. Twenty-four choroidal melanoma patients who were treated with a carbon ion beam underwent at least three MET-PET scans before and after therapy. The uptake was visually and semiquantitatively evaluated on the basis of the tumor-to-brain ratio (TBR). We found accumulation was significantly decreased at 6 months or more after therapy and disappeared in 50% of the patients at 12 months after therapy. The baseline TBR, 1, 6, 12 and 24 months after therapy averaged 1.88±0.65, 1.73±0.52, 1.08±0.42, 0.67±0.27 and 0.65±0.30, respectively. TBR was significantly decreased at 6 months or more after therapy. It was concluded that MET-PET could be an alternative method for evaluating the effect of radiotherapy.

2) Image Processing Research Team

We quantified intrafractional organ motions due to respiration in the thoracic site as a function of time using the 256 multi-slice (MS) CT. Patients were immobilized on the patient bed, as routinely used in treatment. After several minutes rest in a supine
position on the CT bed, all 4D CT acquisitions were performed under free breathing, with patient respiration monitored by the respiratory sensing system. Scan conditions were slice collimation of 256 x 0.5 mm or 128 x 1.0 mm, with 0.5 s in a single rotation and scan time of less than 6 s to obtain one respiratory cycle without patient couch movement. The respiratory cycle was subdivided into 10 phases, with $T_p$ as peak inhalation and $T_e$ as peak exhalation. Gross tumor volume (GTV) was manually contoured on the CT data set at peak exhalation by a certified radiation oncologist. GTV contours at other phases were calculated by deformable registration, following which the oncologist checked the contour curves at each phase. Center of mass (COM) was calculated by using the GTV contours. The GTVs are displayed as a function of time in Fig. 3-5.

Fig. 3-5. Lung sagittal images at (a) peak exhalation, (b) mid-inhalation and (c) peak inhalation. The red lines show the GTV.

For interfractional analysis, gas in the bowel and stomach could also cause dose variation. However, it is relatively difficult to acquire 4D CT data in the abdominal region due to the limitation of patient radiation dose. Therefore, triple phase dynamic enhancement CT acquisitions were routinely acquired for diagnostic purposes under inhalation breath-holding using a 16MSCT. After an initial scout topogram and non-enhanced CT scan (native phase) of the abdomen, CT acquisitions were generated in all patients in the helical mode. CT acquisition was started at 35 s (arterial phase), 70 s (venous phase), and 180 s (delayed phase) after injection. The scan interval times of the venous phase and delayed phase from the arterial phase were 35 s and 145 s, respectively. We defined the arterial phase CT data (scan interval time 0 s) as a treatment planning CT and calculated the compensating bolus, which was then applied to the CT data sets at the other two phases.

Figure 3-6 shows carbon ion dose distributions at the scan interval times of 0, 35 and 145 s in axial and coronal sections (patient no.1). Since the bolus was designed to cover the CTV at the planning CT, over 95% of the dose was delivered to the CTV at 0 s. Although anatomical positions at each phase were similar, beam overshoot/undershoot was observed at 35 and 145 s due to extension/shortening of the radiological path length from the anterior and left directions against the planning CT. However, total prescribed dose is not so much a problem even though it causes dose variation due to gas bowel movement.

Fig. 3-6. Carbon ion beam distributions in axial and coronal sections (patient no. 1). Times of (a) 0 s (planning CT), (b) 35 s and (c) 145 s. Beam overshoot (yellow arrows) and undershoot (white arrows) were observed at the scan interval times of 35 and 145 s. Green and yellow lines show GTV and CTV contours, respectively. Red, pink, light blue and blue lines show 95%, 80%, 50% and 30% of doses, respectively.

We evaluated intrafractional organ motion and dose variation due to interfractional change. Our approach as described here needs to have uncertainties for each treatment planning process quantified in order to provide solutions for increased treatment accuracy. We are convinced, however, that our approach to moving targets in charged particle therapy will be a decisive factor in overcoming these problems and in improving treatment.

3) Quality Control Research Team

Due to frequent radiotherapy accidents, the importance of quality control in radiotherapy has been increasingly recognized. The Quality Control Research Team tries to meet the expectations for safe and reliable radiotherapy through dosimetric research. NIRS has been the Secondary Standard Dosimetry Laboratory (SSDL) for radiotherapy in Japan. The NIRS standard ionization chambers have been calibrated in terms of $^{60}$Co exposure by the National Metrology Institute of Japan (NMJJ). More than 700 therapy-level dosimeters from hospitals were calibrated with the NIRS $^{60}$Co standard exposure field in the last fiscal year. The team has prepared the standard field of absorbed dose to water and tried to establish measurement traceability in terms of absorbed dose to water in collaboration with NMJJ. The code of practice for therapeutic dosimetry is being revised.

After the establishment of the nation-wide dosimetry audit system using glass dosimeters last year, the team carried out dosimetry intercomparison between this system and the IAEA audit system which is using TLDs (thermoluminescence dosimeters). The results showed a good agreement within 1% for the average dose. This audit system was also applied to the dosimetry intercomparison with Asian countries within the framework of the Forum for Nuclear Cooperation in
Asia (FNCA). China, Korea, Indonesia and Viet Nam had participated in the intercomparison by 2008. The audit detected cases of overexposure with approximately 6% of the dosimeters and recommended the MU value be calibrated correctly.

The Quality Control Research Team has also carried out the studies with regard to dosimetry for therapeutic hadron beam.

The team conducted a nation-wide proton dosimetry intercomparison which involved new proton facilities in Japan. The results showed a good agreement within 0.4% and established there was dose uniformity among the domestic proton facilities.

The team developed a graphite calorimeter for absolute absorbed dose measurements. The graphite calorimeter was applied to the determination of w-value of air for the therapeutic carbon beam. The w-value was evaluated as 35.72 J/C ± 1.5%, which is 3.5% larger than that recommended by the IAEA code of practice for heavy ion beams.

From the viewpoint of microdosimetry, tissue-equivalent proportional counters (TEPCs) were used to study the estimation of clinical dose at HIMAC. The RBE values for carbon beams were calculated by the microdosimetric kinetic model (MKM) and spectra measured with the TEPCs. The field size dependence of the clinical dose was obtained from TEPC measurements.

These research activities are expected to influence other radiotherapy facilities in Japan as well as NIRS itself. We also intend to contribute to the field of radiotherapy internationally in cooperation with organizations such as the IAEA, WHO and the International Organization for Standardization (ISO).

4) Radiological Protection Research Team
   a) Dose estimation and protection against medical radiation

Recently, more X-ray CT screenings for early detection of lung cancer have been done because of the usefulness of their image information and relatively lower doses. Most persons undergoing CT screenings are healthy, so the optimization of CT scan conditions is very important. We have measured organ doses of patients participating in the CT screening using an adult anthropomorphic phantom and TLDs under CT scan conditions routinely used at two hospitals. The estimated equivalent doses of thyroid, lung, esophagus, breast, liver and stomach were between 0.8 and 2.6mGy. Based on evaluation of image quality for the CT images as well as estimated doses, adequate CT scan conditions were suggested that consider the optimization of radiation protection in CT screenings for lung cancer.

In addition to the exposures to patients, the occupational exposures of medical radiation workers in brachytherapies were also estimated. TLDs were attached on the clothing and exposed skin surfaces of a physician who performed 125I brachytherapy for prostate cancer treatments, and surface doses during the therapies were directly measured. The results showed that the left hand and arm were higher dose positions of the body, which were nearer to the 125I seeds and the X-ray beam in fluoroscopy. The doses could be reduced to less than 100 μSv when the physician used lead gloves for the protection of the hands. They could also be decreased due to increased consciousness of the physician regarding the exposures and when the physician became more experienced.

To consider radiation safety in proton radiotherapy and CIRT, their secondary neutron doses were measured in five domestic therapy institutes by using neutron rem-counters and water phantoms. Then the results were compared among institutes. The measured doses varied and differences between proton beams and carbon ion beams were observed. The neutron ambient dose equivalents in CIRT were lower than those of proton radiotherapy. To specify the positions producing neutrons, Monte Carlo simulations were made and concrete methods to reduce the exposures of patients to secondary neutrons were studied based on the calculated data. The validations of simulated data by actual measurements are in progress.

   b) Survey of medical exposure

A nation-wide survey concerned with X-ray examinations was done by sending questionnaires to about 1,500 hospitals and clinics. The sampled facilities were chosen after categorizing medical facilities into 5 groups depending on their bed numbers. The facilities were requested to supply data on exposure conditions and frequencies in both diagnoses and fluoroscopy in X-ray examinations. The reply data have been analyzed to estimate the total annual frequency and population dose of X-ray examinations by gender and age group. The data on X-ray CT examinations in the survey performed last fiscal year have also been analyzed.

Major publications


3) S. Mori, K. Nishizawa, C. Kondo, M. Ohno, K.


3.4. RadGenomics Project for Radiotherapy

Takashi Imai, Ph.D.
Director, RadGenomics Research Group

(Outline of Research Career)

Dr. Imai received a Ph.D. from the University of Tsukuba in 1986. Following completion of a fellowship from the Japan Society for the Promotion of Science for Japanese Junior Scientists at the Institute of Applied Biochemistry, University of Tsukuba, he joined the Tsukuba Life Science Center, Institute of Physical and Chemical Research (RIKEN). From 1988 to 1989, he worked in the Department of Genetics, Washington University Medical School (St. Louis, Missouri, USA) as a visiting research associate. Here Professor Maynard Olson sparked his interest in the human genome project. After joining The Cancer Institute, (Japanese Foundation for Cancer Research) in 1991, Dr. Imai worked on cancer and population genomics. He moved to NIRS in 1994. From 2001 to 2006, he worked as the project leader of the RadGenomics Project. Since 2006 he has been the director of the RadGenomics Research Group.

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**Objectives**

Normal tissue reactions of cancer patients vary considerably after radiotherapy. A number of observations have indicated that certain genetic factors play important roles in this variability. The aim of the RadGenomics Research Group is to explore the genetic characteristics of both the patient and the bearing tumor, by which the potentially most effective radiotherapy can be delivered. From the molecular-biological standpoint, this will open the way to the development of an individual-oriented radiotherapy. The project will also contribute to future research on the molecular mechanisms of radiation sensitivity in humans.

**Progress of Research**

1) **Patients**

Between October 2001 and March 2009, 2,653 patients were registered including 773 breast cancer patients, 345 cervical cancer patients, 855 prostate cancer patients, and 271 head and neck cancer patients. Normal tissue reactions until the third month after completion of the treatment were graded according to the National Cancer Institute-Common Toxicity Criteria (NCI/CTC). Late effects on normal tissues were graded according to the Radiation Therapy Oncology Group/ the European Organization for Research and Treatment of Cancer (RTOG/EORTC) scoring system and the Late Effects of Normal Tissues-Subjective, Objective, Management and Analytic (LENT-SOMA) scoring system. Patients were divided into two groups (radiosensitive and radioresistant) according to the grades determined by the above scoring systems.

2) **Influence of multiple genetic polymorphisms on genitourinary morbidity after carbon ion radiotherapy for prostate cancer**

Objective: To investigate the genetic risk of late urinary morbidity after carbon ion radiotherapy in prostate cancer patients.

Methods and Materials: A total of 197 prostate cancer patients who had undergone carbon ion radiotherapy were evaluated for urinary morbidity. The distribution of patients with dysuria was as follows: Grade 0, 165; Grade 1, 28; and Grade 2, 4 patients. The patients were divided (2:1) consecutively into the training and test sets and then categorized into control (Grade 0) and case (Grade 1 or greater) groups. First, 450 single nucleotide polymorphisms (SNPs) in 118 candidate genes were genotyped in the training set. The associations between the SNP genotypes and urinary morbidity were assessed using Fisher’s exact test. Then, various combinations of the markers were tested for their ability to maximize the area under the receiver operating characteristics (AUC-ROC) curve analysis results. Finally, the test set was validated for the selected markers.

Results: When the SNP markers in the SART1, ID3, EPDR1, PAH, and XRCC6 genes in the training set were subjected to AUC-ROC curve analysis, the AUC-ROC curve reached a maximum of 0.86. The AUC-ROC curve of these markers in the test set was 0.77. The SNPs in these five genes were defined as “risk genotypes.” Approximately 90% of patients in the case group (Grade 1 or greater) had three or more risk genotypes.

Conclusion: Our results have shown that patients with late urinary morbidity after carbon ion radiotherapy can be stratified according to the total number of risk genotypes they harbor.

3) **In-gel multiple displacement amplification of long DNA fragments diluted to the single molecule level**

Objective: The isolation and multiple genotyping of long individual DNA fragments are needed to obtain haplotype information for diploid organisms.

Methods and Materials: A limited amount of cellular DNA was carefully released from intact cells into a mildly heated alkaline agarose solution and mixed thoroughly. The solution was then gently aliquoted and allowed to solidify while maintaining the integrity of the diluted DNA. Exogenous provided Phi29 DNA polymerase was used to perform consistent genomic amplification with random hexamer oligonucleotides within the agarose gels. Simple heat melting of the gel allowed recovery of the amplified materials in a solution of the polymerase chain reaction (PCR)-ready form.

Results: The haplotypes of seven SNPs spanning 240 kb of the DNA surrounding the human ATM gene region on chromosome 11 were determined for 10 individuals.

Conclusion: The newly developed ignment technique described herein, used in combination with the previously established visible multiple SNP typing array, allows convenient experimental haplotype determination with ordinary laboratory instruments. Currently, this method can be used to determine effectively the haplotypes of loci that contain multiple markers, and it allows precise mapping of genes for low numbers of samples such as for individual patients.

4) **Visible genotype sensor array**

Objective: To develop a visible sensor array system for simultaneous multiple SNP genotyping using a new plastic base with specific surface chemistry.

Methods and Materials: Discrimination of SNP alleles was carried out by an allele-specific extension reaction using immobilized oligonucleotide primers. The 3'-ends of oligonucleotide primers were modified with a locked nucleic acid to enhance their efficiency in allelic discrimination. Biotin-dUTPs included in the reaction
mixture were selectively incorporated into extending primer sequences and were utilized as tags for alkaline phosphatase-mediated precipitation of colored chemical substrates onto the surface of the plastic base.

Results: The visible precipitates allowed immediate inspection of typing results by the naked eye and easy recording by a digital camera equipped on a commercial mobile phone. Up to four individuals were analyzed on a single sensor array and multiple sensor arrays were handled in a single operation. All of the reactions were performed within one hour using conventional laboratory instruments.

Conclusion: This visible genotype sensor array is suitable for "focused genomics" that follows "comprehensive genomics".

5) Aging-dependent large accumulation of muscle-specific point mutations in the transcription/replication control region of human mitochondrial DNA

Objective: The aging-dependent large accumulation of specific point mutations, especially the most frequent mutation T414G, in the cultured human skin fibroblast mtDNA transcription/replication regulatory region raised the question of their occurrence in post-mitotic tissues.

Results: Analysis of biopsied or autopsied human skeletal muscle from various aged individuals revealed the absence or only minimal presence of those skin fibroblast mutations. By contrast, surprisingly, most of the 26 individuals 53 to 92 years old, without a known history of neuromuscular disease, exhibited at the same region of human mtDNA in muscle an accumulation of two new point mutations, i.e., A189G and T408A, which were absent or marginally present in the muscle of 19 individuals younger than 34 years. These two mutations were not found in the skin fibroblasts from 22 subjects 64 to 101 years of age (T408A), or were present only in three subjects in very low amounts (A189G). Furthermore, in several older individuals exhibiting an accumulation in muscle of one or both of these mutations, they were nearly absent in other post-mitotic tissues, whereas the most frequent fibroblast-specific mutation (T414G) was present in skin autopsy, but not in muscle.

Conclusion: The striking tissue specificity of the aging-dependent mtDNA point mutations and their mapping at critical sites for mtDNA transcription/replication strongly point to the involvement of a specific mutagenic machinery or a specific advantage for the mtDNA replication/transmission and to the functional relevance of these mutations during the human aging processes.

6) Expression profiles are different in carbon ion-irradiated normal human fibroblasts and their bystander cells

Objective: Evidence has accumulated that ionizing radiation induces biological effects in non-irradiated bystander cells having received signals from directly irradiated cells; however, energetic heavy ion-induced bystander response is incompletely characterized. Then, microarray analysis of irradiated and bystander fibroblasts in confluent cultures were carried out.

Materials and Methods: Each of 1, 5 and 25 sites was targeted with 10 carbon ions (18.3MeV/u, 103keV/mum) using microbeams. Cultures were exposed to 10% survival dose (D), 0.1D and 0.01D of corresponding broadbeams (108keV/mum). Irrespective of the target numbers (1, 5 or 25 sites) and the time (2 or 6h post-irradiation), similar expression changes were observed in bystander cells.

Results: Among 874 probes that showed more than 1.5-fold changes in bystander cells, 25% were upregulated and the remainder downregulated. These included genes related to cell communication (PIK3C2A, GNA13, FN1, ANXA1 and IL1RAP), stress response (RAD23B, ATM4 and EJF2AK4) and cell cycle (MYCN, RBBP4 and NEUROG1). Pathway analysis revealed serial bystander activation of G protein/P1-3 kinase pathways. However, genes related to cell cycle or death (CDKN1A, GADD45A, NOTCH1 and BCL2L1) and cell communication (IL1B, TCF7 and ID1) were upregulated in irradiated cells, but not in bystander cells.

Conclusion: The results indicate different expression profiles in irradiated and bystander cells, and imply that intercellular signaling between irradiated and bystander cells activates intracellular signaling, leading to the transcriptional stress response in bystander cells.

7) CD44 and Bak expression in IL-6 or TNF-alpha gene knockout mice after whole lung irradiation

Objective: To understand the molecular mechanisms that underlie radiation pneumonitis, we examined whether knockout of the TNF or the IL-6 gene could give mice an inherent resistance to radiation in the acute phase of alveolar damage after thoracic irradiation.

Methods: The temporal expression of inflammation (CD44) and apoptosis (Bak) markers in lung after thoracic irradiation was measured to determine the degree of alveolar damage.

Results: At 4 weeks post-irradiation (10 Gy), small inflammatory foci were observed in all mice, but there were no obvious histological differences between control (C57BL/6JSlc), TNF-alpha knockout (TNF KO), and IL-6 knockout (IL-6 KO) mice. However, immunohistochemical analysis of CD44 and Bak expression over a time course of 2 weeks highlighted significant differences between the three groups. C57BL/6JSlc and TNF KO mice had increased numbers of both CD44-positive and Bak-positive cells after irradiation, while the IL-6 KO mice showed stable
levels of CD44 and Bak.

Conclusion: The radioreistant status of IL-6 KO mice in the acute phase of alveolar damage after irradiation suggests an important role for IL-6 in radiation pneumonitis.

8) The proangiogenic factor ephrin-a1 is upregulated in radioreistant murine tumor by irradiation

Objective: While the pre-treatment status of cancer is generally correlated with outcome, little is known about microenvironmental changes caused by anti-cancer treatment and how they may affect outcome. We attempted to find a gene that was both induced by irradiation and associated with radioreistance in tumors.

Methods and Materials: Using singlecolor oligoarrays, we analyzed the gene expression profiles of two murine squamous cell carcinomas, NR-S1, which is highly radioreistant, and SCCVII, which is radiosensitive, after irradiation with 137-Cs gamma rays or carbon ions. Candidate genes were those differentially regulated between NR-S1 and SCCVII after any kind of irradiation. Four genes, Efna1 (Ephrin-A1), Sprtl (small proline-rich protein 1A), Srgap3 (SLIT-ROBO Rho GTPase activating protein 3) and Xnr1 [RIKEN 2 days neonate thymus thymic cells (NOD) cDNA clone E430023D08 39], were selected as candidate genes associated with radiotherapy-induced radioreistance. We focused on Efna1, which encodes a ligand for the Eph receptor tyrosine kinase known to be involved in the vascular endothelial growth factor (VEGF) pathway. We used immunohistochemical methods to detect expression of Ephrin-A1, VEGF, and the microvascular marker CD31 in radioreistant NR-S1 tumor cells.

Results: Ephrin-A1 was detected in the cytoplasm of NR-S1 tumor cells after irradiation, but not in SCCVII tumor cells. Irradiation of NRS1 tumor cells also led to significant increases in microvascular density, and up-regulation of VEGF expression.

Conclusion: Our results suggest that radiotherapy-induced changes in gene expression related with angiogenesis might also modulate microenvironment and influence responsiveness of tumors.

9) Dose measurement on both patients and operators during neurointerventional procedures using photoluminescence glass dosimeters.

Objective: Although radiation skin injuries associated with interventional radiology are known to be a critical issue, there are few reports mentioning direct measurement of the entrance skin dose (ESD). Thus, the purpose of this study was to clarify the regional distributions of ESDs in neurointervention.

Materials and Methods: Using photoluminescence glass dosimeters (PLDs), we measured the ESDs in 32 patients with a median age of 61.5 years. Angiographic parameters, including exposure time, dose-area product (DAP), and the number of digital subtraction angiography (DSA) studies and frames, were recorded. The ESDs of operators were analyzed by the same method.

Results: The maximum ESD of 28 therapeutic procedures was 1.8 +/- 1.3 Gy. Although the averaged ESD on the right temporoo-occipital region was higher than that in other regions, disease-specific patterns were not observed. Statistically positive correlations were found between the maximum ESD and exposure time (r = 0.3283, P = .005), DAP (r = 0.7917, P < .001), the number of DSA studies (r = 0.5636, P = .002), and the number of DSA frames (r = 0.8583, P < .001). As for operators, ESDs to the left upper extremity were significantly higher than those to other regions. However, most of the ESDs were <0.2 mGy. Lead protective garments reduced the exposure doses to approximately one half to one tenth.

Conclusion: The regional ESD can be measured by applying the PLD. This method should contribute to reducing the dose accumulation in patients as well as in operators.

Major publications


Ryuichi Okayasu Ph.D.
Director, Heavy-ion Radiobiology Research Group

(Outline of Research Activities)

Dr. Okayasu received his Ph.D. in radiation biology from Colorado State University, USA in 1987 and worked as a post-doctoral fellow at Thomas Jefferson University, Philadelphia and MD Anderson Cancer Center, Houston. He next took a position at Columbia University as an associate research scientist before moving to the University of Texas Medical Branch at Galveston in 1995 as an Assistant Professor and then onto Colorado State University. In 2002, he moved back to Japan to become a team leader at the International Space Radiation Laboratory (ISRL), NIRS. In 2005 he was appointed as Director of ISRL. In 2006, he changed his section was transferred to the Research Center for Charged Particle Therapy and became Director of the Heavy Ion Radiobiology Research Group.

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Objectives
There are three mid-term plans for the Heavy Ion Radiobiology Research Group. Plan 1 has one goal: to provide biological experimental data for analyzing clinical data with regard to tumor control ratio and normal tissue responses for various radiation therapy protocols. Plan 2 has two goals: to estimate the risk and benefit ratio between tumor cell killing and normal tissue sparing by theoretical calculations based on patients’ dose distribution as well as experimental data on cell and animal studies; and to propose a more efficient radiation therapy regimen by comparing heavy ion radiotherapy and other radiotherapy protocols such as use of X-rays. Plan 3 has four goals: to explore radiosensitizers and protectors which can be used with heavy ion radiotherapy; to elucidate the mechanism of effective heavy ion treatment for hypoxic tumor cells which show strong resistance to radiation; to study the indirect (bystander) effects of radiation which occur in non-irradiated cells adjacent to irradiated cells; and to integrate the proposals of Plan 2 to improve radiation therapy and accumulate biological data resources for a new cancer therapy.

These objectives are studied by four teams: 1) Biophysics Team; 2) Experimental Therapy Team; 3) Cellular and Molecular Biology Team; and 4) Radiation Modifier Team. Each team has different objectives, however, cooperation among four teams is sought in order to accomplish the goals of the group.

Progress of Research
Biophysics Team
To estimate relative biological effectiveness (RBE) for unknown ion beams at a defined linear energy transfer (LET), an experimental fitting function of the LET-RBE relationship was proposed. Experimentally obtained LET-RBE spectra of survival curves for V79 cells exposed to ³He-, ¹²C-, ¹⁰Ne, ²³Si-, ²⁴Ar, and ⁶⁰Fe-ion beams with the LET range of 10-500 keV/µm were applied for the study; the exposures were done at the HIMAC, the Medical Cyclotron, and the RRC/RIKEN. Cell survival curves were fitted by an equation of the LQ-model to obtain survival parameters (a and b). The RBE spectrum was analyzed as a function of LET for each ion beam using the proposed function with three parameters: Lp, A, and W. The respective parameters indicate a LET that gives the maximum RBE, a related value to maximum RBE, and the width of the peak of RBE. Clear splits of the LET-RBE spectra were found among ion species. It was also found that those parameters can be defined as functions of atomic numbers (or atomic mass numbers) of the accelerated ion beams. The LET that gave the maximum RBE shifted to higher LET region, and the maximum RBE value decreased with increasing atomic number. The width of the peak was constant when the atomic mass number was smaller than 20, but it increased when the mass number was greater than 20. This method is applicable to estimate overall RBE in therapeutic beams. This is because the beam must consist of different ions having different RBEs that are produced by projectile fragmentation of the beam when passing through a patient’s body or absorbing materials when adjusting beam range in the body. The biological endpoint for this study was limited to the cell survival at 10% for one cell line. It is necessary to analyze different biological endpoints and different cell lines. We are continuing to study the LET-RBE spectra for different cell lines, DNA damage and cell killing by direct or indirect action of radiation, mutation and transformation for different ion beams at various LETs.

Experimental Therapy Team
Our data confirmed that the RBE values by carbon ions at three different LET values 15, 45, 75 keV/µm were 0.6, 1.0 and 1.4 respectively when calculated at 20% tumor formation frequency of irradiated mice. Furthermore, we have an ongoing research study examining the biological effect of the ratio of cells with two distinct radiosensitivities in vivo. Towards this end, in vitro colony assay data have been applied.

To investigate the relationship between LET and skin reaction, we have performed fractionated monopeak irradiation on the normal mouse foot. The α/β ratios were 28 and 39 Gy for LET values of 58 and 14 keV/µm, respectively, and 38 Gy for γ rays. There seems to be no significant difference among the α/β ratios.

In addition, we have found that carbon-ion irradiation can curatively eradicate transplantable human colon cancer, which showed radioresistance to conventional X-rays, and the suppression of tumor-induced angiogenesis and the disruption of cancer stem cells were considered to be crucial molecular mechanisms of heavy ion radiotherapy. The RBE value for carbon ions (relative to X-rays) for in vivo tumor control was 3.82.

Cellular and Molecular Biology Team
Biological differences between X-ray and heavy ion particle (e.g., C, Fe, Ne) irradiations were demonstrated using some quantitative assays such as immune staining with phosphorylated proteins and chromosome aberrations, focusing on the molecular mechanism for the early stage of DNA damage response at therapeutic level radiation doses. Using several radiosensitive mutant cell lines, we aimed at understanding the role of either homologous recombination or non-homologous end joining on the mechanism under the repair process of DNA double-strand breaks induced by various
heavy-ion particles.

HiCEP, a novel comprehensive gene expression technique developed in NIRS, was applied to normal human fibroblasts which were irradiated with X-rays and carbon ion particles at a dose of 2 Gy. A group of early responsive IR-induced genes (ATF3, BTG2, TP53INPI) remained activated for a longer period in human cells irradiated with carbon ion particles when compared with conventional X-rays. Our team, for the first time, revealed that the expression of ASPM, a microcephaly gene was significantly downregulated by IR in human and murine cells.

**Radiation Modifier Team**

This team has studied three subjects. Biosynthetic precursors of vitamin E are known to have a substituted p-hydroquinone structure. In order to develop better compounds for free radical scavenger, a kinetic study for free radical scavenging reaction of vitamin E precursors and their derivatives was carried out. The second-order rated constants determined by the stopped-flow technique suggested that the electron-donating groups on the benzene ring of p-hydroquinones significantly enhance the free radical scavenging activity. Regarding the second subject, radiation-protectors and radiation-mitigators, several compounds that had been found to be effective against low LET radiation-induced injury were found to protect or mitigate bone marrow death of mice induced by carbon ion radiation, too. As the third subject, the distribution of anti-cancer drugs into the brain was visualized using nitroxyl labeled anti-cancer drug and 7T MRI in a collaboration with the Molecular Imaging Center. A new data acquisition method for CW EPR spectral-spatial imaging was proposed. Details of reactions between nitroxyl radical and glutathione were investigated. Depth-dependent free radical generation in gelatin sample caused by irradiating a carbon ion mono beam was detected with a nitroxyl contrast agent using EPR spectroscopic and MRI methods.

**Major publications**


3.6. Transcriptome Research for Radiobiology

Masumi Abe, Ph.D.
Director, Transcriptome Research Group

(Outline of Research Activities)

The Transcriptome Research Group, consisting of three teams, Stem Cell Research Team, Gene Expression Profiling Team, and Model Organism Research Team, pursues transcriptome research for radiobiology. Contact point: abemasum@nirs.go.jp
Objective

Transcriptome Research Group, consisting of three teams, Stem Cell Research Team, Gene Expression Profiling Team, focuses on and Model Organism Research Team, the effect of radiation at an individual level not at a cellular level only.

Progress of Research

1) Stem Cell Research Team

This team has been focused on germ stem cells. It is known that several genes are expressed both in embryonic stem cells (ESCs) and germ stem cells. However, their function in germ stem cells is still unknown. Our team identified a new gene that expresses in both ESCs and spermatogonial stem cells (SSCs). We generated its knockout mice and found a severe defect in their spermatogenesis, resulting in an accumulation of SSCs in the mice. Further study revealed that the gene plays a role in the differentiation step of SSCs.

Recently the team is also conducting a new project on iPS cells. It has been demonstrated that somatic cells can be converted into pluripotent stem cells by ectopic expression of four genes, Oct3/4, Klf4, Sox2 and cMyc genes, designated as iPS cells. The objective of this program is to understand the molecular mechanism underlying iPS generation. First, we attempted to observe the emergence of iPS cells from somatic cells. To this end, we developed a new investigation system by improving a pre-existing time-lapse system. Consequently, we made the first successful detection of the conversion process of a fibroblast into a stem cell. Quite interestingly such cell lineage conversion occurred within 3 days after the defined gene infection. Furthermore, by means of the HiCSEP (High Coverage gene Expression Profiling) method, which has been developed by our "Gene Expression profiling Team" as mentioned below, we isolated the genes which are closely related in iPS emergence from Day 3 fibroblasts, in which approximately one per 2,000 fibroblasts were converting into stem cells. Currently, we are focusing on their functions.

2) Gene Expression Profiling Team

The HiCSEP method that we have developed is an ideal tool for transcriptome analysis, and it is based on a principle different from that for hybridization-based methods. HiCSEP technology enables us to achieve comprehensive analysis of transcripts including novel ones.

This year we attempted to improve the HiCSEP method to achieve the analysis by using even a small amount of starting materials. At the beginning of HiCSEP development, approximately 1 \( \mu \)g of poly (A) RNA was needed for the analysis; however, subsequent improvement has allowed us to perform the analysis with a total RNA amount of 0.1 \( \mu \)g which corresponds to 10,000 mammalian cells. This year we successfully developed a new protocol using 200 pg of total RNA, corresponding to 20 of mammalian cells.

Applying this new protocol of the HiCSEP method to single cell transcriptome analysis, which contains approximately 10 pg of total RNA, we could observe only about 10,000 transcripts, almost all of which were highly expressed. Now we are attempting to develop an analysis using fewer than 10 cells with high coverage detection.

Using HiCSEP technology, we are also beginning a new program for medical applications. The ethical committee of NIRS has authorized our proposal, and we have set up an area and system that will allow us to perform the HiCSEP analysis on human materials. The first trial will be started soon.

3) Model Organism Research Team

This team has been primarily supporting other research teams, especially the Stem Cell Research Team. They have generated TG mouse for one gene and KO mice for two genes.

In addition, they are studying spermatogenesis using the spermatogonial stem cell transplantation technology. Functional analysis on iPS cells were also performed by this team.