3. Research Center for Charged Particle Therapy

Tadashi Kamada, MD, Ph.D.
Director, Research Center for Charged Particle Therapy

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 30 years of experience in clinical research on radiation oncology, including 14 years of experience in carbon ion radiotherapy at the 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 Research Center for Charged Particle Therapy, NIRS since 2008.

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**Objectives**

The Research Center for Charged Particle Therapy (hereafter, abbreviated as "Center") was established in 1998 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 experience of carbon ion radiotherapy in various types of malignant tumors, the Center was successful in obtaining approval from the Ministry of Health, Welfare and Labor for "Highly Advanced Medical Technology" in 2003. Thus carbon ion therapy has meanwhile achieved for itself a solid place in general practice of cancer treatment. The HIMAC has been also served for >500 researchers as a multi-user utilization facility for medical, biological and physics research.

In 2006, when the second Mid-Term 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 the 2008 fiscal year include: a 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 SNPs; research on a HICEP (High Coverage Gene Expression Profiling) system.

**Overview**

The Center is organized into 6 research groups for two major topics (A and B) and one invited research project (C). Progress of research for each topic is summarized next.

A. Research on the use of heavy ion beams for cancer radiotherapy.

i. Development of advanced cancer radiotherapy with charged particles

This subject has been carried out by the Particle Therapy Research Group (GL: H. Tsutji) consisting of 3 teams: the Clinical Trial Research Team, the Clinical Database Research Team, and the Radiation Effect Research Team.

According to the long-term objectives, research on developing advanced clinical therapy using carbon ion beams has been aggressively performed in FY 2009 as well as in previous years.

The Clinical Trial Research Team has succeeded in maintaining a large number of patients per year. Specifically, 682 patients, the maximum number ever, underwent carbon ion radiotherapy (C-ion RT) in FY 2009. So far, a total of 5196 patients were enrolled in clinical trials of C-ion RT. Prostate, lung, head and neck, bone and soft tissue, and liver tumors were the leading 5 tumor types in the trials.

The outcomes of clinical trials revealed that 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, mainly in the treatment of common cancers, such as lung cancer (single fraction), liver cancer (two fractions), and prostate cancer. New clinical trials of combined treatment of C-ion RT and chemotherapy were started to obtain even better survival outcomes in intractable tumors, such as pancreatic cancer, brain tumors, and malignant melanoma of head and neck regions. Survival benefits in malignant melanoma by combined treatment has been already observed.

Improvement of efficiency in C-ion RT is also an important subject in the effective use of a limited capacity of the facility. A new method for manufacturing range compensators, which ensures preparation of the treatment, has been developed and used in actual treatments. Furthermore, the setup procedure could become easier and faster with a new field localization system using a flat panel detector (FPD), which was also began to be used this year.

The Clinical Database Research Team has 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). The developed information systems, conforming to the Integrating the Healthcare Enterprise (IHE), Enterprise User Authentication (EUA) and Patient Synchronized Applications (PSA) functions, made it easy to operate multiple systems in one clinical unit. As a result, the developed system contributed to improved efficiency of patient registration and resulted in an increase in the number of patients. In addition, the processing speed and ease of operation of the clinical database system have been improved. By using this system physicians can analyze patients by the heavy particle radiation therapy protocol and generate survival curves in a few seconds.

The Radiation Effect Research Team has aggressively performed experiments and analyses as well.

In order to analyze the mouse skin reaction in fractionated C-ion radiation, applicability of the LQ
model was investigated together with the RCR (repairable-conditionally repairable) model and the multi-target two components model. While the LQ model failed to express a decrease in response by single irradiation, RCR showed good agreement with experimental observations.

In addition to research on verification of actual radiation field, which was performed last year, the MKM (Microdosimetric Kinetic Model) was applied to estimate cell survival of hypoxic cells based on the response underoxic conditions in FY 2009. As a result, it was found that by adjusting the domain size to half that ofoxic conditions, the cell survival of hypoxic cells could be correctly estimated.

2 Development of a novel irradiation system for charged particle therapy

This subject has been carried out by the Medical Physics Research Group (GL: K. Noda) consisting of 4 teams: the Accelerator Development Research Team, the Irradiation System Research Team, the Therapy System Research Team, and the Compact Heavy-Ion Therapy System Research Team.

On the basis of more than 10 years of experience with HIMAC, the Medical Physics Research Group has designed and constructed a new treatment research facility toward "adaptive cancer therapy" with heavy ions, which makes the oneday treatment of lung cancer possible. Furthermore, the new treatment research facility should accurately treat a fixed target, a moving target with breathing and/or a target near to a critical organ. For these purposes, a 3D-scanning method with a pencil beam is employed. A phase-controlled rescanning (PCR) method has been proposed and studied, especially for treating a moving target. In the new treatment facility, a rotating gantry with the PCR method will be also employed in order to reduce the patient's load, and to increase the treatment accuracy for a tumor near to a critical organ through the multifield optimization method. After the design of beam-optics, a mechanical design has been carried out. As a result, the weight of the gantry is suppressed to 350 tons, which is almost half that of HIT gantry. For multifield optimization, inverse-planning has been further studied. It was verified that the method can reduce the dose in OAR significantly while keeping that in the target.

Including the studies mentioned above, we have designed fixed beam-delivery, rotating-gantry, treatment-management, patient-positioning and treatment planning systems in the new treatment research facility. Related R&D work has also been carried out with HIMAC since April 2006. A building of the new treatment research facility was completed in March 2010. After installing the devices of the beam transport and beam-delivery systems, a beam commissioning and pre-clinical study are scheduled in FY 2010.

3 Standardization and improvement of therapeutic and diagnostic techniques

This research covers a wide range of research and has been performed by the Diagnosis and Treatment Advancement Research Group (GL: T. Kanada) consisting of 4 teams: the Image Diagnosis Research Team, the Image Processing Research Team, the Quality Control Research Team, and the Radiological Protection Research Team.

The Image diagnosis research team studied two PET tracers, $^{68}$Cu-ATSM and C-11- Methionine, for onologic imaging. This year, tumor hypoxic imaging using $^{68}$Cu-ATSM for cervical cancer was continued and metastatic lymph node imaging using C-11-Methionine (MET) was also investigated. For Cu-62-ATSM imaging for tumor hypoxia, this team found that accumulation of squamous cell carcinoma in C-62-ATSM PET/CT after CIRT was significantly lower than pre-therapeutic accumulation. This might imply that squamous cell carcinoma of the uterine cervix tended to hypoxic in pre-therapeutic condition and that CIRT might improve its hypoxic condition. The improvement of hypoxic condition might be associated with the therapeutic effect of CIRT. For metastatic lymph node imaging using C-11-methionine, this team found that MET-PET/CT was useful for diagnosis of neck lymph node metastasis and especially specificity was relatively high. There were very few true positive metastases in neck lymph node accumulation in the MET-PET/CT study from trunk cancers compared to head and neck cancers. But diagnostic capability for neck lymph node metastasis from trunk cancers was higher than from head and neck cancers.

The Image processing research team analyzed intrafractional organ movement during respiration using 4D CT (256MSCT) applied to patients with pancreatic carcinoma in 2009. They evaluated intrafractional organ motion and dose validation for un gated and gated treatments. Doses to organs at risk were smaller in the gated than in the un gated treatment, although the differences were small. They suggest that un gated pancreatic treatment may deliver a sufficient accumulated dose through the treatment course with minimal dose variation due to respiratory pattern variation, and in this regard is therefore preferable to the gated treatment. The use of an un gated treatment may shorten total treatment duty time by a factor of three compared with gated treatment in pancreatic cancer.

The quality control research team has developed a graphite calorimeter for absolute absorbed dose
measurement. The absorbed dose obtained by the calorimeter was approximately 3 to 4% higher than that by an ionization chamber for carbon beams. The disparity seems to arise from uncertainties of stopping power and the w-value for carbon beams.

This team carried out studies on dosimetry for therapeutic hadron beams.

The radiological protection research team performed dosimetric studies for secondary cancer risk after receiving carbon-ion and proton radiotherapies. Absorbed dose, quality factor and dose equivalent in water phantom outside of the irradiation field were determined by microdosimetric measurements with a commercial tissue equivalent proportional counter at passive carbon-ion and proton radiotherapy facilities: HIMAC and National Cancer Center Hospital East. They confirmed that the total secondary doses per treatment in carbon-ion and proton radiotherapies were comparable to or less than those in 3D-CRT and IMRT. They were considerably less than those in 3D-CRT and IMRT as the position became closer to the field edge. Verification of Monte-Carlo simulations, which is needed to assess the detailed distributions of dose and biological effectiveness, is in progress.

B. Research on radiation effects for improvement of radiation therapy

1. RadGenomics research concerning the radiation sensitivity

This subject has been carried out by the RadGenomics Research Group (GL; T Imai) consisting of 3 teams: the Genetic Information Team, the Molecular Radio-oncology Team, and the 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 a 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 radiosensitivity of patients having undergone radiotherapy. In FY 2009, we focused on the following research subjects.

First, we investigated a novel molecular biomarker for cervical adenocarcinoma (AD) through the integration of multiple methods of genomic analysis. A difference between biopsy samples of AD and squamous cell carcinoma (SCC) was identified in the expression and genomic copy number of Villin1 (VILL). Kaplan-Meier survival curves revealed worse disease-free survival in VILL-positive tumors. The marker was validated by 65 newly enrolled patients, and VILL positive staining showed 52% sensitivity and 100% selectivity for cervical AD. This study suggests the existence of a subtype of cervical tumors which are VILL positive with a poor radioreponse.

Next, we extended our previous finding that fibroblast growth factor 2 (FGF2) expression levels in tumor cells (FGF2-T) may be an indicator of the efficacy of radiotherapy in cervical cancer (CC), using newly enrolled patients and further investigated stromal FGF2 expression, which was detected in tumor cells of all cases and in stromal cells in 87% of cases. Radiation causes a response in tumor cells and adjacent normal cells, and changes the extracellular matrix environment. In this study, we confirmed our previous findings showing that changes in FGF2-T expression may be used as a marker to monitor the effectiveness of radiotherapy for CC. Our findings should improve patient selection for molecular targeted therapies, such as cytokine inhibitors, following standard-of-care treatment.

Finally, to clarify how carbon-ion radiotherapy (C-ion) on primary tumors affects the characteristics of subsequently arising metastatic tumor cells, mouse squamous cell carcinomas, NR-S1, syngenic C3H/HeNsr mice were irradiated with C-ion or gamma-rays. Irradiation doses used in this study did not suppress primary tumor growth, but inhibited lung metastasis significantly. We found no difference in the incidence and histology, and only small differences in expression profile, of distant metastasis between local C-ion and gamma-ray radiotherapy. The application of local radiotherapy per se or the type of radiotherapy applied did not influence the transcriptional changes caused by metastasis in tumor cells.

2. Biological research concerning the improvement of radiation therapy

This subject has been carried out by the Heavy-Ion Radiobiology Research Group (GL; R Okayasu) consisting of 4 teams: the Biophysics Team, the Experimental Therapy Team, the Cellular and Molecular Biology Team, and the Radiation Modifier Team.

Biophysics Team: In order to clarify the contribution of indirect and direct effects induced by heavy ions, cell survival fractions were measured for various LET values (15 to 480 KeV/μm) using Chinese hamster ovary (CHO) cells. The contribution of direct action
increased as LET increases; the contribution of indirect action was about 30% when LET was 480 keV/μm. The relative biological effectiveness (RBE) was determined for direct action (RBED) and indirect action (RBEI) separately. The maximum RBED was 9.1 at 480 keV/μm and the maximum RBEI was 2.6 at 90 keV/μm. These results indicate that the direct action induced by heavy ions results in a very high biological effectiveness.

**Experimental Therapy Team:** Malignant melanoma showed a good local control by heavy ion treatment despite the low overall survival of patients. The effects of carbon ions (C-ions) on metastatic potential were studied for melanoma in vitro and in vivo. Carbon-ion showed higher cytotoxic effects on B16/B16L6 cells in vitro than X-rays. Both migration and invasion potential of cells were enhanced by photon beams at low doses (0.5 to 1 Gy) when compared to non-irradiated controls; however, these factors were suppressed by C-ions. The RBE values for migration and invasion in vivo were higher than that for cell killing. The number of lung metastatic nodules after tumor-irradiations decreased with each dose, and C-ions were more effective than photons. Our study suggests that C-ion significantly inhibits the metastatic process when compared with low-LET photons. This team also studied the effects of stem cells in tumors irradiated with heavy ions using a tumor xenograft model. Significant control of stem-like cells was observed after C-ion irradiation.

**Cellular and Molecular Biology Team:** We developed a useful chordoma cell line, U-CH1-N out of the only chordoma cell line available in the world, and determined its radio- and chemosensitivity. Our data provides the first chronological cell survival using cells of the chordoma origin and helps to explain the successful chordoma treatment by heavy ions. A group of early responsive IR-induced genes (e.g., ATF3, BTG2, TP53INP1) remained activated in human cells irradiated with carbon ions when compared with X-rays. We found that the expression of ASPM, a microcephaly gene, was significantly downregulated by IR in human and murine cells. We started to characterize the function of this gene by RNA interference; a significant increase in radiosensitivity of several tumor cell lines was demonstrated. The other approach was to generate a mouse model whose Aspm orthologous gene (calbpm1) was conditionally disrupted.

**Radiation Modifier Team:** To develop a new radioprotector, we measured the redox potential of various natural antioxidants. We found a new radioprotector in an in vivo setting having nitroxyl radical and edaravone moieties. We measured the DPF value of γ-TMG against X-irradiated bone marrow death of mice; it was 1.2 by i, p. administration of the compound immediately after exposure. The effect of radioprotectors on tumor regulation by heavy ions was investigated using a mouse xenograft model. The effect of amnionyte on tissue oxygen tension was measured by EPR oximetry. The distribution of reactive oxygen species generated by heavy ions was also measured and analyzed. In addition, the combination of X-ray and a PI3-kinase inhibitor was found to enhance anti-tumor activity both in vitro and in vivo.

**3 Transcriptome Research for Radiobiology**

This subject has been carried out by the Transcriptome Research Group (GL; Abe) consisting of 3 teams: the Stem Cell Research Team, the Gene Expression Profiling team, and the Model Organism Research Team.

This year these teams obtained some notable results, especially on induced pluripotent stem (iPS) cells and on HiCEP technology as follows.

Recently, it has been demonstrated that somatic cell can be converted into pluripotent stem cells by ectopic expression of four genes, Oct3/4, Klf4, Sox2 and cMyc. Such somatic cell reprogramming suggests the possibility of generating patient-specific pluripotent stem cells. Replacement of or adding tissues prepared from patient-specific stem cells have great potential for the therapeutics of radiation-induced injuries. While needless to say, elucidating the molecular mechanisms underlying iPS generation is a key issue for efficient preparation of safe iPS cells that can be use for various medical uses, it has been quite difficult due to its unique characteristics, extremely low efficiency and stochastic manner.

The Transcriptome Research Group attempted to observe the emergence of iPS cells from somatic cells directly. Finally, they developed a new investigation system by improving a pre-existing time-lapse system that allows us to precisely investigate iPS generation at short intervals over 2 weeks. Using this system, they first succeeded in directly observing the conversion process of a somatic cell into a stem cell. Interestingly, it was revealed that the onset of the cell lineage conversion already initiated within 48 hours just after the defined gene infection in most (85.7%) iPS cell generations. In addition, unexpectedly, no morphological asymmetric cell division occurred during the conversion process from an ancestral somatic cell into an iPS cell. Namely, ancestral fibroblast cells gradually transformed into stem cells with several symmetric cell divisions. Thus, they provided critical new insight during the first three days of iPS cell generation, which was completely unknown.

This group also made another contribution in the field of iPS. They succeeded in generating genome integration-free iPS cells without an oncogene, c-Myc, for the first time, by transduction. In addition, these
iPS cells were established from somatic cells prepared from inbred mice whose genome sequence was completely determined. These materials, therefore, will serve as a valuable resource for future genetic studies of iPS cell generation.

Meanwhile, this team has developed an ideal transcriptome analysis procedure called High coverage gene expression profiling (HiCEP). The method is based on a principle different from various hybridization-based methods. This year they improved the HiCEP method to allow it to analyze even a small amount of starting materials, less than 10-20 mammalian cells. On the other hand, they also developed a new effective system for HiCEP analysis. They have developed a high throughput machine for HiCEP reaction called HiCEPer that enables us to analyze more than 15,000 samples per year, and a precision PCR (polymerase chain reaction) machine for the HiCEP reaction. This equipment was released in the H21 fiscal year. In addition, they developed a HiCEP reaction kit, for 1 μg starting material. The kit allows anyone, even without expertise in molecular biology, to achieve HiCEP analysis.

C. Research Projects with Heavy Ions at NIRS-HIMAC

131 proposals were accepted and carried out in FY2009 at HIMAC. 5490 hours of beam time was supplied in that research. 108 papers and 39 proceedings were published, while 331 papers were presented at various meetings. A total of 625 researchers participated in the project, including 85 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 27 years of experience in clinical research on radiation oncology, including 14 years experience in carbon ion radiotherapy at the 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**

- 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.
- A study on optimizing irradiation methods by disease and by region, using clinical investigations of therapies in which radiation is combined with drugs and operations.
- Development of a comprehensive database on treatment, clinical course and other factors. Comparison and analysis of domestic and foreign data on particle beam therapy.
- Annual treatment of 650 patients to maximize and disseminate the therapeutic effect of charged particle technology. This is the target number combining patients taking part in clinical studies and those receiving high-technology treatments, in consideration of the fact that the NIRS is primarily a research and development facility.
- Evaluation of the therapeutic effects of treatments developed by the NIRS from the viewpoint 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, the Clinical Database Research Team, and the Radiation Effect Research Team. All teams are performing 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 2010, a total of 5196 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.

![Tumor sites in Carbon ion radiotherapy at NIRS](image)

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

We treated 692 new patients in FY 2009. Prostate, lung, head and neck, bone and soft tissue, and liver tumors are the 5 major tumor types in the trials. A total of 4604 patients who had a follow-up period of 6 months or more were included in this report. Clinical trials revealed that carbon ion radiotherapy provided definite local control and offered a survival advantage with acceptable 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. Nearly 74% of the patients receiving carbon ion radiotherapy were treated by HAMT in 2009.

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. We developed a new MLC with thinner leaves and proved that the leakage dose of the MLC was comparable to the present MLC. Therefore, it is possible to use the new MLC for more precise field shaping without a patient-collimator; however, it is necessary to design a new treatment control system prior to installing the new MLC into the beam line for the actual patient treatment. A new treatment control system has already started to be designed that would also be available for treatment in the new facility.

Range compensators are also essential in the broad beam method. A new method for manufacturing range compensators, employing a punch technology, has been developed. 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 takes half an hour or less. The range compensators made with this new method has started to be used in actual treatment.

The new field localization system using a flat panel detector (FPD) was started to be used in 2009. Since localization images with FPD have higher resolution than conventional radiographs, the setup procedure could become easier and faster than ever.

2) Clinical Database Research Team

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

We also developed information systems that conform to the Integrating the Healthcare Enterprise (IHE) Enterprise User Authentication (EUA) and Patient Synchronized Applications (PSA) functions. These functions make it easy to operate multiple systems. Two PCs (EMR and PACS viewer) are commonly used for the Hospital Information System in one clinical unit. Physicians have to enter a user ID and password to log into these systems. The IHE-EUA and PSA function to 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 RM and Web Service Description Language (WSDL) and HL7. We prepared the Open Source Software license for the delivery of software. We implemented the document source, document repository, document registry and document consumer that were defined by the IHE XDS. We had developed the application software. We are now designing and developing interface function that communicates between the existing system such as EMR and/or PACS and the IHE XDS system. We think that it is very important to establish a new IHE integration profile. This enables the Treatment Management System to receive and send radiotherapy orders.

We have a clinical database system which contains information concerning over 5,200 patients with heavy particle radiation therapy and over 22,800 patients with photon radiation therapy. We improved the processing speed and ease of operation of this database system. By using this system physicians can analyse patients by the heavy particle radiation therapy protocol and generate survival curves in a few seconds. The clinical database can manage data concerned with the disease history, staging, radiation schedule, radiation dose/days, adverse effects and follow-up information.

The NIRS Hospital Information System in May 2010 is shown in Fig. 3-2.

Fig. 3-2. Current configuration of Hospital Information System in NIRS.

3) Radiation Effect Research Team

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 \( \alpha/\beta \) value of the GU was 7.7, more than 2 times larger than that against photons (3.0) in the literature. BED calculated with the \( \alpha/\beta \) value for carbon-ion beam was 73.8, which was consistent with that for photons, 74.7. The information will contribute to the prospective estimation of a prescribed dose in different fractionation or further dose optimization in treatment planning.

Reaction of skin is one of the most important endpoints to be considered in radiotherapy; however,
its analysis from clinical outcomes is not easy as radiation quality and dose given to patients significantly differs individually. From this point of view, skin reaction has been investigated through the reaction observed on mice. Through the fractionated irradiation of carbon beams to mouse leg, it was found that the effect of a single fraction irradiation differs uniquely from that by multiple fractionations: the efficacy tends to be small on single fractions. In order to analyze the response, the applicability of a commonly-used LQ model was investigated together with the RCR (repairable-conditionally repairable) model and the multi-target two components model. While the LQ model failed to express a decrease in response by single irradiation, RCR showed good agreement with experimental observations.

In addition, we have started a fractionated cell irradiation experiment with carbon ions by adjusting the time gap between irradiations from 0 to 120 min in order to clarify the initial repair of damage in order to understand clinical outcomes.

Lineal energy information measured by a tissue-equivalent proportional counter in the therapeutic irradiation field was found to be useful for estimating biological effectiveness of the beam at a point by processing the information with the Microdosimetric Kinetic Model (MKM). This year, the method has been applied for the verification of actual irradiation fields and the following results have been obtained.

Field effect

It was found that, in the case of a small irradiation field, the decrease in absorbed dose at the center of the irradiation field by a collimator is almost well compensated by the increase in radiation quality. The resulting isoeffective dose is regarded to be 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 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; it was confirmed that the therapeutic beam provided in each port can be regarded as identical.

Oxygen effect

MKM was applied for the estimation of cell survival of hypoxic cells based on the response under oxic conditions. It was found that by adjusting the domain size to half of that for oxic conditions, the cell survival of hypoxic cell was correctly estimated.

Major Publications

5. Takeshi Yanagi, Tadashi Kamada, Hiroshi Tsuji, Reiko Imai, Itsuko Serizawa, Hirohiko Tsuji: Dose-volume histogram and dose-surface histogram analysis for skin reactions to carbon ion radiotherapy for bone and soft tissue sarcoma, Radiotherapy and Oncology
3.2. Development of a Precise Irradiation System for Heavy-ion Therapy

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

Outline of Research Career:
Dr. Koji Noda received his B.S. degree from the Department of Nuclear Engineering, Kyushu University in 1979. After completing the M.S. programs there in 1981, he worked for the development of a PET cyclotron from 1981 to 1989, and he also studied the accelerator physics from 1984 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 Director of the Department of Accelerator and Medical Physics, and he holds the additional post of Director of the Medical Physics Research Group.

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**Objectives:**

On the basis of more than 10 years of experience with HIMAC, we have designed and constructed a new treatment research facility toward "adaptive cancer therapy" with heavy ions, which makes the one-day treatment of lung cancer possible. Further, the new treatment research facility should accurately treat a fixed target, a moving target with breathing and/or a target near to a critical organ. For these purposes, a 3D-scanning method with a pencil beam is employed in this project. 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 increase the treatment accuracy for a tumor around in the vicinity of critical organ through the multi-field optimization method, while reducing the patient's load. 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. Related R&D works have also been carried out with HIMAC since April 2006. Building of the new treatment research facility was completed in March 2010. After installing the devices of the beam transport and beam-delivery systems, a beam commissioning and preclinical study will be carried out in FY 2010.

**Progress of Research:**

1) **Planning of the new treatment research facility**

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

By cooperating with medical staff in the HIMAC hospital, the treatment hall has been designed. 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 an X-ray CT. Further, six rooms are devoted to patient preparation before irradiation. The facility building was completed in March 2010, as shown in Fig. 3-3.

Specifications of the facility are summarized in Table 1.

**Table 1. Specification of the new treatment facility**

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<th>1. Basic parameters</th>
<th>2C, 30 (2C, 30)</th>
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<tr>
<td>Delivery beam intensity</td>
<td>107 - 109 pps at 2C</td>
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<td>Treatment room</td>
<td>2 fixed-beam rooms (Horizontal&amp;Vertical), 1 rotating-gantry room</td>
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<tr>
<th>2. Fixed beam-delivery system</th>
<th>140 - 400 MeV/n</th>
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<tr>
<td>Energy</td>
<td>140 - 400 MeV/n</td>
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<tr>
<td>Irradiation method</td>
<td>Fixed target: 3D raster scanning with pencil beam</td>
</tr>
<tr>
<td>Moving target: PCR method</td>
<td>Moving target: PCR method</td>
</tr>
<tr>
<td>Scanning speed</td>
<td>H: 100 mm/ms, V: 50 mm/ms</td>
</tr>
<tr>
<td>Spot size</td>
<td>2 - 4 mm at 1-sigma</td>
</tr>
<tr>
<td>Lateral-field/SOBP/Range size</td>
<td>22 cm in square/15 cm/ &gt; 25 cm at 2C</td>
</tr>
<tr>
<td>Irradiation-port length</td>
<td>9 m</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Rotating-gantry system</th>
<th>Iso-centric rotating gantry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>140 - 400 MeV/n</td>
</tr>
<tr>
<td>Irradiation method</td>
<td>Same as the fixed beam-delivery system</td>
</tr>
<tr>
<td>Scanning speed</td>
<td>H: 100 mm/ms, V: 50 mm/ms</td>
</tr>
<tr>
<td>Spot size</td>
<td>2 - 4 mm at 1-sigma</td>
</tr>
<tr>
<td>Lateral-field/SOBP/Range size</td>
<td>15 cm (15 cm/15 cm/ &gt; 25 cm at 2C)</td>
</tr>
<tr>
<td>Displacement of iso-center</td>
<td>&lt; 1 mm</td>
</tr>
<tr>
<td>Size and weight</td>
<td>Length: 16.5 m, Radius: 7.1 m, Weight: 350 tons</td>
</tr>
</tbody>
</table>
Fig. 3-3. Schematic view of the HIMAC and the new treatment facility with photograph of the new building completed (Left). The completed building of the new treatment research facility.

2) Related R&D work

a) Development of accelerator technology

An extended flattop operation of the HIMAC synchrotron was successfully developed, which can shorten the irradiation time by a factor of 2 even under rescanning. This operation method has been routinely utilized in the raster rescanning experiment. Owing to the new treatment planning, this operation mode and the high speed scanning magnet, the irradiation speed has been increased by around 100 times more than the conventional spot scanning.

In the present beam-scanning system, a range shifter, consisting of PMMA plates of various thicknesses, is used to degrade the beam energy and to control the depth dose-distribution. When using the range shifter, the setting time of the range shifter takes almost the same time as the irradiation time. Furthermore, since focused pencil beams will be used in the raster-scanning irradiation, this range shifter may broaden the spot size of the beam on the target, and also produce secondary fragments, which would adversely affect the depth dose-distribution. Therefore, it is preferable to change the beam energy directly by accelerators instead of using a range shifter. To change the beam energy, as extracted from the synchrotron ring, we propose a multiple-energy operation with the quasi-DC extension of flattops. The proposed operation enables us to provide heavy ions having variable energies within a single synchrotron-cycle; namely, the beam energy would successively change more than 100 times within a single synchrotron-pulse by an energy step, corresponding to a water range of 2 mm. With this operation, the beam range would be controlled without using energy degraders, such as the range shifter, and hence an excellent depth dose distribution could be obtained. The scheme of the multi-energy operation and the preliminary experimental results are shown in Fig. 3-4 (a) and (b), respectively.
Fig. 3-4 (a) A schematic drawing of an operation pattern for the synchrotron ring. The operation pattern has a stepwise flattop, each of which can be extended, and the beam can be extracted from the synchrotron ring during any of these flattops. 
(b) A current pattern for the sextupole magnets (yellow), the DCCT in the ring (blue), and the measured beam current of the extracted beam (green). The beam energy of the extracted beam is 379.5 MeV/u.

b) Experiment of fast 3D raster scanning
A test irradiation port was designed and installed to a HIMAC physics-experimental line in order to experimentally verify the fast 3D raster-scanning and the PCR method. This test port has the same configuration as the fixed beam-delivery system in the new treatment facility, as shown in Table 1 and Fig. 3-3. The scanning experiment has been carried out since December 2008. In the experiment, the extended flattop operation has been routinely utilized. As a result of the experiment, it was verified that the scanning speed with the designated value was achieved without disturbing the dose distribution. Using a moving phantom we carried out an irradiation experiment on the moving target. As the result, we obtained and verified a uniform dose distribution with the fast 3D rescanning.

Major publications:
3) M. Kumagai, S. Mori, S. Gregory, H. Asakura, S. Kandatsu, M. Endo, M. Baba: Dosimetric Variation Due to CT Inter-Slice Spacing in Four-Dimensional Carbon Beam Lung Therapy, Physics in Medicine and Biology, 54 (10), 3231-3246, 2009.
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 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.
- Research and development 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, the image processing research team, the quality control research team and the radiological protection research team, and performs research into the advancement and standardization of radiation therapy and diagnostic methods. The progress of research in each team is summarized.

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 have been assessing whether Cu-62 labeled diacetyl-bis(N-(4-methylthiosemicarbazone) (Cu-62-ATSM) imaging of tumor hypoxia is associated with C-11-methionine imaging of amino acid metabolism in cervical cancer. Our data showed that cervical cancer had a greater tendency to incorporate C-11-Methionine than Cu-62-ATSM before any treatment. Pre-therapeutic accumulation of both Cu-62-ATSM and C-11-Methionine showed a significant difference between squamous cell carcinoma and other tumor groups. But post-therapeutic accumulation of either Cu-62-ATSM or C-11-Methionine showed no significant difference between squamous cell carcinoma and other tumor groups. Accumulation of squamous cell carcinoma in Cu-62-ATSM PET/CT after CIRT was significantly lower than pre-therapeutic accumulation. It might imply that squamous cell carcinoma of uterine cervix tended to be hypoxic in pre-therapeutic conditions and CIRT might improve its hypoxic condition. Improvement of the hypoxic condition might be associated with the therapeutic effect of CIRT.

Cervical cancer had a greater tendency to incorporate C-11-Methionine than Cu-62-ATSM before any treatment.

Studies using C-11 methionine with PET have been undertaken for some study subjects. We performed a study about the diagnostic capability of C-11 methionine PET/CT for neck lymph node metastasis from head and neck cancers versus trunk cancers. In this study, we evaluated the detectability of MET-PET/CT for neck lymph node metastasis from head and neck tumors or from the other primary origin trunk tumors. We reviewed MET-PET/CT images of 1749 studies, from June 2006 to February 2007, searching for any nodular accumulation in the neck area. We selected patients with any nodular accumulation in the neck area as suspicious candidates for lymph node metastasis and we evaluated diagnostic indexes and made ROC curve analyses. We concluded that MET-PET/CT was useful for diagnosis of neck lymph node metastasis; specificity in particular was relatively high. There were very few true positive metastases in neck lymph node accumulation in the MET-PET/CT study from trunk cancers compared to head and neck cancers. But the diagnostic capability for neck lymph node metastasis from trunk cancers was higher than from head and neck cancers.

We examined the usefulness of C-11 methionine PET/CT for predicting recurrence, metastasis and prognosis of patients with lung cancer treated by carbon ion radiotherapy. PET/CT was performed before and after CIRT for each patient. Post-therapeutic PET/CT was performed at 1 month or 3 months after CIRT completion. The tumor to normal tissue ratio (TNR) before and after CIRT, the result of recurrence, the result of systemic metastasis and the result of prognosis were entered into the Kaplan-Meier analysis. Our data showed that patients with high TNR before CIRT had a significantly higher recurrence rate and poorer prognosis than patients with low TNR. Patient with high TNR at 3 months after CIRT had a significant poorer prognosis than patients with low TNR. TNR at 1 month after CIRT did not show any statistically significant relation to recurrence, metastasis or prognosis. There was no significant relation between TNR and incidence of metastasis. We concluded that MET uptake in lung cancer was a successful predictor of recurrence and survival. TNR at 3 months after CIRT is a better predictor for prognosis than TNR at 1 month after CIRT.

Regarding the usefulness of methionine, PET was also used to evaluate the response evaluation and predicting prognosis of uterine cervical cancer treated by carbon-ion beam radiotherapy. We evaluated the relationship between C-11 methionine (MET) uptake and clinical outcome such as local recurrence, systemic...
metastasis and prognosis. ECAT EXACT HR+ and ECAT EXACT 47 PET scanner (Siemens CTI, Knoxville, TN) were used for PET imaging in this study. Pre-and post-therapeutic tumor TNR, its change after CIRT, the rate of local recurrence, the rate of systemic metastasis and prognosis were entered into the Kaplan-Meier analysis. Our data showed that the intensity of uptake of squamous cell carcinoma had a higher tendency than that of adenocarcinoma, although there was no significant difference between them. Pre-therapeutic TNR had a statistically significant relationship with local recurrence, systemic metastasis and prognosis. Post-therapeutic TNR was significantly related to recurrence and prognosis, and TNR residual ratio after CIRT was significantly related to metastasis and prognosis. In the squamous cell carcinoma group, pre-therapeutic TNR had a statistically significant relationship to metastasis and prognosis. In the adenocarcinoma group, pre-therapeutic TNR was significantly related only to recurrence. We concluded that PET-PET was a successful predictor for local recurrence, systemic metastasis and prognosis in patients with uterine cervical cancer treated by CIRT. But the relationship depended on the histological type of cervical cancer.

2) Image processing research team

We quantified pancreatic tumor motions due to respiration by using the 256 multi-slice CT. Patients were immobilized on a bed with, as routinely performed in treatment. CT scans were performed under free breathing, with patient respiration monitored by the respiratory sensing system. Scan conditions were slice collimation of 1.28 x 1.0 mm, 0.5 s in a single rotation and a 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 T0 as peak inspiration and T30 as peak exhalation. Gross tumor volume (GTV) and clinical target volume (CTV) 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 Figure 3-5.

![Figure 3-5](image)

Figure 3-5 Four-dimensional sagittal CT images. (a) T0 (peak inspiration), (b) T20, (c) T40, (d) T50 (peak exhalation), (e) T70 and (f) T90. The yellow line and blue mesh grid show the GTV contours and deformed space from peak exhalation, respectively.

To compare respiratory-gated and respiratory-un gated treatment strategies using 4DCT datasets, we evaluated 4D scattered carbon ion beam distribution in the pancreatic region. Two types of compensating bolus were designed for respective CTVs to cover the whole and periresection CTV moving regions, which defined the 30% duty cycle around exhalation. The carbon ion beam dose distribution was calculated as a function of the respiratory phase by applying the compensating bolus to 4DCT at the respective phase. The accumulated dose distribution was calculated by registering the carbon ion beam distribution at the respective phases to that at peak exhalation (T50) by applying deformable registration, which creates transformation maps.

Figure 3-6 shows accumulated carbon ion dose distribution for the ungated and gated treatments. We calculated the difference in accumulated dose distribution between the gated and ungated treatment by subtracting the accumulated dose distribution for the gated treatment from that for the ungated treatment (Fig. 3-6c). Large positive dose differences (over 10%) were observed mainly on the inferior aspect, resulting from the fact that gated treatment irradiates only during exhalation phases. Doses to organs at risk were smaller in the gated than the ungated treatment, although the differences were small.

Given that our results for ungated and gated pancreatic treatment were closely similar with regard to tolerance doses to normal tissues, and that doses were less than the tolerance dose in both ungated and gated treatments, and taking into account the above problem, we suggest that ungated pancreatic treatment may deliver a sufficient accumulated dose through the treatment course with minimal dose variation due to
respiratory pattern variation, and in this regard is therefore preferable to gated treatment. The use of an ungated treatment may shorten total treatment duty time by a factor of three compared with the gated treatment.

Figure 3-6 Accumulated carbon ion beam dose distribution for (a) ungated treatment, (b) gated treatment, and (c) accumulated dose distribution differences (ungated minus gated) (Patient 1). Axial (upper row), coronal (middle row), and sagittal (lower row) sections. White areas, yellow areas, and dark green lines show the planning target volume (PTV), clinical target volume (CTV), and gross tumor volume (GTV) contours, respectively. Red, green, pink, light blue, and blue lines show 95%, 80%, 70%, 50%, and 30% of total doses, respectively.

We evaluated intrafractional organ motion and dose validation for ungated and gated treatments. Our approaches described here are necessary to quantify uncertainties for each treatment planning process and provide solutions for increasing 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 of NIRS tries to meet the expectations for safe and reliable radiotherapy mainly 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 60Co exposure by the National Metrology Institute of Japan. More than 700 therapy-level dosimeters from hospitals were calibrated with the NIRS 60Co standard field in the last fiscal year. The team is preparing to establish the standard field of absorbed dose to water and has calibrated the NIRS standard chambers in terms of absorbed dose to water, in collaboration with the International Atomic Energy Agency (IAEA).

To establish a nation-wide dosimetry audit system in radiotherapy, the team carried out comparative studies between the glass dosimeters and TLD which had been used as a postal dosimeter. The results showed that the glass dosimeters were appropriate for the postal dose audit with their features. The team carried out a pilot study in which postal glass dosimeters were sent to approximately 100 hospitals in Japan. The pilot study showed 1.3% standard deviation of dose among the 100 hospitals. Since November 2007, a regular dosimetry audit service for radiotherapy facilities has been started using a glass dosimeter with a commercial base by the Association for Nuclear Technology in Medicine, in collaboration with the National Cancer Center and NIRS. The team still continues to carry out studies with glass dosimeters for non-reference conditions in therapeutic X-rays.

In addition, the team has carried out studies on dosimetry for hadron therapy. The team conducts nation-wide dosimetry intercomparison with all hadron therapy facilities in Japan, including the Gunma University Heavy Ion Medical Center which started carbon beam therapy in March 2010. The consistency of doses has been determined with a standard deviation of 0.5%. The team has also developed a graphite calorimeter for absolute absorbed dose measurements. The absorbed dose obtained by the calorimeter was approximately 3 to 4% higher than that by an ionization chamber for carbon beams. The differences seem to arise from uncertainties of stopping power and the w-value for the carbon beams.

These research activities are expected to contribute to other radiotherapy facilities as well as the NIRS. The quality control research team also intends to contribute to the field of radiotherapy internationally in cooperation with organizations such as the Forum for Nuclear Cooperation in Asia (FNCA), the IAEA, the World Health Organization (WHO), the International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC).

4) Radiological protection research team

a) Dose estimation and protection against medical radiation

An increase in the frequency of CT examinations over the past decade has raised concerns about radiation doses and the possible detriment to the health of children. The estimation of accurate dose levels and radiation risks requires organ doses for pediatric patients in CT examinations. We have evaluated organ doses in head CT, chest CT and abdomino-pelvic CT scan conditions routinely used at 23 hospitals with TLDs or photodiode dosimeters implanted at various
tissue and organ positions within a 6-year-old child anthropomorphic phantom. In head CT scans, organ doses for brain were 20-49 mGy except for one case. In chest CT and abdominopelvic CT scans, organ doses within scan ranges were 2-21 mGy and 3-21 mGy, respectively, which were on average approximately 20-50% lower than in adult chest CT and abdominopelvic CT. Organ doses varied among CT protocols mainly due to differences in the types of CT scanners and effective mAs. The setting of proper effective mAs and a strict scan length could reduce the doses in pediatric CT examinations. The dose data evaluated in this study would be useful for the evaluation of dose levels and radiation risks for children and would also lead to the optimization of pediatric CT scan protocols.

The secondary cancer risk after receiving carbon-ion and proton radiotherapies has become a great concern because of positive outcomes of the radiotherapies. Such exposure is considerably lower than that near the treatment volume, but it is not negligible for estimating the risk, especially for young patients. Organ-specific dosimetric data in the patient is essential for assessing the risk, but experimental data are scarce. Therefore, absorbed dose, quality factor and dose equivalent in water phantom outside of the irradiation field were determined by microdosimetric measurements with a commercial tissue equivalent proportional counter at passive carbon-ion and proton radiotherapy facilities: HIMAC and the National Cancer Center Hospital East. We confirmed that the total secondary doses per treatment in carbon-ion and proton radiotherapies were comparable to or less than those in 3D-CRT and IMRT, and especially, they were considerably less than those in 3D-CRT and IMRT as the position became closer to the field edge. Verification using Monte-Carlo simulations, which are needed to assess the detailed distributions of dose and biological effectiveness, is in progress.

Major Publications

1) Shinichiro Mori, Ryusuke Hara, Takeshi Yanagi, Sharp Gregory, Motoki Kunagai, Hiroshi Asahara, Riwa Kishimoto, Shigeru Yamada, Susumu Kandatsu, Tadashi Kamada: Four-dimensional Measurement of Intrafractional Respiratory Motion of Pancreatic Tumors Using a 256-Multislice CT Scanner, Radiotherapy and Oncology, 92, 231-237, 2009

2) Shinusuke Yonai, Naruhito Matsufuji, Tatsuaki Kanai: Monte Carlo study on secondary neutrons in passive carbon-ion radiotherapy: Identification of the main source and reduction in the secondary neutron dose, Medical Physics, 36 (10), 4830-4839, 2009,


4) A. Fukumura; H. Tsuji; T. Kamada; M. Baba; H. Tsuji; H. Kato; S. Kato; S. Yamada; S. Yasuda; T. Yanagi; H. Kato; R. Hara; N. Yamamoto; J. Młodek; K. Akahane; S. Fukuda; Y. Furusawa; Y. Iwata; T. Kanai; N. Kanematsu; A. Kitagawa; N. Matsufuji; S. Minohara; N. Miyahara; H. Mizuno; T. Murakami; K. Nishizawa; K. Noda; E. Takeda; S. Yonai. CARBON-ION RADIOTHERAPY: CLINICAL ASPECTS AND RELATED DOSIMETRY, Radiation Protection Dosimetry 127, 149-155, 2009

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 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 at 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 a 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) **Study population**

Between October 2001 and March 2010, 2782 patients were registered including 775 breast cancer patients, 409 cervical cancer patients, 896 prostate cancer patients, and 310 head and neck cancer patients. Normal tissue reactions until the 3rd 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/ 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 radioreistant) according to the grades determined by the above scoring systems.

2) **Application of carbon-ion beams or gamma-rays on primary tumors does not change the expression profiles of metastatic tumors in an in vivo marine model.**

**Objective:** To clarify how carbon-ion radiotherapy (C-ion) on primary tumors affects the characteristics of subsequently arising metastatic tumor cells.

**Methods and Materials:** Mouse squamous cell carcinomas, NR-S1, in syngenic C3H/HeMsnrs mice were irradiated with a single dose of 5-50 Gy of C-ion (290 MeV per nucleon, 6-cm spread-out Bragg peak) or γ-rays (137Cs source) as a reference beam. The volume of the primary tumors and the number of metastatic nodules in lung were studied, and histologic analysis and microarray analysis of laser-microdissected tumor cells were also performed.

**Results:** Including 5 Gy of C-ion and 8 Gy of γ-rays, which did not inhibit the primary tumor growth, all doses used in this study inhibited lung metastasis significantly. Pathologic findings showed no difference among the metastatic tumor nodules in the nonirradiated, C-ion-irradiated, and γ-ray-irradiated groups. Clustering analysis of expression profiles among metastatic tumors and primary tumors revealed a single cluster consisting of metastatic tumors different from their original primary tumors, indicating that the expression profiles of the metastatic tumor cells were not affected by the local application of C-ion or γ-ray radiotherapy.

**Conclusion:** We found no difference in the incidence and histology, and only small differences in the expression profile, of distant metastasis between local C-ion and γ-ray radiotherapy. The application of local radiotherapy per se or the type of radiotherapy applied did not influence the transcriptional changes caused by metastasis in tumor cells.

3) **Villin1, a novel diagnostic marker for cervical adenocarcinoma.**

**Objective:** The number of new cervical adenocarcinoma (AD) cases has risen slowly; however, its histological similarity to other tumor types and the difficulty of identifying the site of the original tumor makes the diagnosis of cervical AD particularly challenging. We investigated a novel molecular biomarker for cervical AD through the integration of multiple methods of genomic analysis.

**Methods:** Tumor samples in discovery set were obtained from 87 patients who underwent radiotherapy, including 31 cervical AD. Microarray analysis and quantitative polymerase chain reaction analysis were performed to screen a candidate diagnostic molecule for cervical AD, and its clinical significance was investigated by immunohistochemical analysis (IHC).

**Results:** We found a difference between biopsy samples of AD and squamous cell carcinoma (SCC) in the expression and genomic copy number of Villin1 (VIL1), which maps to 2p35. IHC revealed 14 VIL1-positive tumors; 13 cervical AD and 1 small cell carcinoma of the cervix, while no SCCs or endometrial ADs were VIL1-positive. Kaplan-Meier survival curves revealed worse disease-free survival in VIL1-positive tumors. The marker was validated by 65 newly enrolled patients, and VIL1 positive staining showed 52% sensitivity and 100% selectivity for cervical AD.

**Conclusion:** We have identified VIL1 as a novel biomarker of cervical AD. Our study suggests the existence of a subtype of cervical tumors which are VIL1 positive with a poor radiosensitivity.

4) **Change in fibroblast growth factor 2 expression as an early-phase radiotherapy responsive marker in sequential biopsy samples from cervical cancer patients during fractionated radiotherapy.**

**Objective:** We previously showed that fibroblast
growth factor 2 (FGF2) expression levels in tumor cells (FGF2-T) may be an indicator of the efficacy of radiotherapy in cervical cancer (CC). Here, we extended this finding using newly enrolled patients and further investigated the stromal FGF2 expression.

Methods and materials: Sixty-nine patients with CC were recruited as a validation set for the immunohistochemical detection of FGF2-T from biopsy samples taken before (pretreatment) or one week after initiation of radiotherapy (midtreatment). We also investigated the expression of FGF2 in tumor stroma (FGF2-S), and vascular endothelial growth factor (VEGF), and CD31 in these patients plus 35 patients from a previous study.

Results: FGF2 expression was detected in tumor cells of all cases and in stromal cells in 87% of cases. FGF2-T was significantly higher in midtreatment samples ($P = 0.002$), and a high ratio of midtreatment/pretreatment FGF2-T was significantly related to a better prognosis ($P = 0.025$). Increased VEGF expression after initiation of radiotherapy was significantly related to a positive FGF2-S in pretreatment samples ($P = 0.035$), although it was not related to prognosis or microvessel density detected by CD31 expression.

Conclusion: Radiation causes a response in tumor cells and adjacent normal cells, and changes the extracellular matrix environment. In this study, we confirmed our previous findings showing that changes in FGF2-T expression may be used as a marker to monitor the effectiveness of radiotherapy for CC. Our findings should improve patient selection for molecular targeted therapies, such as cytokine inhibitors, following standard-of-care treatment.

5) Vascular homeostasis regulators, Edn1 and Agpt2, are upregulated as a protective effect of heat-treated zinc yeast in irradiated murine bone marrow (Cooperative project with Dr. Anzai, Radiation Modifier Team, Heavy-Ion Radiobiology Research Group).

Objective: To elucidate the mechanism underlying the in vivo radioprotection activity by Zn-containing, heat-treated Saccharomyces cerevisiae yeast (Zn-yeast).

Methods and materials: A Zn-yeast suspension was administered into C3H/He mice immediately after WBI at 7.5 Gy. Bone marrow was extracted from the mice 6 hours after irradiation and analyzed on a microarray. Expression changes in the candidate responsive genes differentially expressed in treated mice were re-examined by qRT-PCR. The bone marrow was also examined pathologically at 6 h, 3, 7, and 14 days post-irradiation.

Results: Thirty-six genes, including Edn1 and Agpt2, were identified as candidate responsive genes in irradiated mouse bone marrow treated with Zn-yeast by showing a greater than three-fold change compared with control (no irradiation and no Zn-yeast) mice. The expressions of Cdc11, Bai1, and Cog3, which are well known radiosensitive genes, were upregulated in WBI mice and Zn-yeast treated whole body irradiation (WBI) mice. Pathological examination showed the newly formed microvessels lined with endothelial cells, and small round hematopoietic cells around vessels in the bone marrow matrix of mice administered with Zn-yeast after WBI, while whole-body irradiated mice developed fatty bone marrow within 2 weeks after irradiation.

Conclusion: This study identified a possible mechanism for the posting irradiation protection conferred by Zn-yeast. The protective effect of Zn-yeast against WBI is related to maintaining the bone marrow microenvironment, including targeting endothelial cells and cytokine release.

Major publications


3.5. Biological Research Concerning the Improvement of Radiation Therapy

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

Outline of Research Career
Dr. Okayasu received his Ph.D. in radiation biology from Colorado State University, USA in 1987. After working as a research scientist at Thomas Jefferson University, MD Anderson Cancer Center, and Columbia University, he became an Assistant Professor at the University of Texas Medical Branch at Galveston in 1995, and then returned to Colorado State University as a faculty. In 2002, he moved back to Japan to become a team leader at the International Space Radiation Laboratory, NIRS. In 2005, he was appointed as Director of ISRL. In 2006, he 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 the 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**

It is believed that the indirect action of radiation plays a less role for high linear energy transfer (LET) radiation when compared with low LET radiation. However, there has not been enough experimental data to support this idea. To clarify the contribution of direct and indirect action of radiation, we obtained experimental data on LET dependencies. The contribution of indirect action mediated by OH radicals in cell killing can be estimated from the maximum degree of protection by applying dimethylsulfoxide (DMSO), which suppresses indirect action without affecting direct action. Exponentially growing Chinese hamster ovary cells under hypoxic condition were exposed to different LET radiation levels from 15 to 480 keV/μm in the presence or absence of DMSO and their survival fractions were determined using a colony formation assay. The contribution of indirect action on cell killing decreased with increasing LET. The contribution was estimated to be 22% at a LET of 480 keV/μm. The relative biological effectiveness (RBE) determined at a survival level of 10% increased with LET, reaching a maximum value of 5.06 at 200 keV/μm, and decreased thereafter. When the RBE was estimated separately for direct action (RBE) and for indirect action (RBE), the RBE was greater than the LET range tested. RBE increased with increasing LET and reached a peak value of 9.10 at 480 keV/μm. RBE showed a peak at 90 keV/μm, but the value was 2.61. Thus the direct action of heavy-ion beams gives a remarkably higher RBE value for cell killing than the indirect action.

**Experimental Therapy Team**

Malignant melanoma showed a high local control at HIMAC, whereas the overall survival of patients was not extended as expected. The control of cancer metastasis is one of the most important issues in cancer treatment. The aim of our study is to clarify the effect of carbon ion beams (C-ions) on metastatic potential of melanoma in vitro and in vivo. Carbon-ion showed higher cytophagic effects on B16/B10 cells in vitro than X-rays. Both migration and invasion potential on cells were enhanced by photon beams at low dose points (0.50 to 1.00 Gy) than non-irradiated controls; however, they were suppressed by C-ions at all dose points tested. The RBE values obtained from migration and invasion tests on cells in vivo were higher than that from cell killing. C-ions significantly suppressed tumor growth. The number of lung metastatic nodules after tumor irradiation decreased with dose, and C-ions were more effective for this than photon beams. The metastatic potentials of survived cells in a tumor after irradiation was analyzed with the number of metastatic lung colonies that formed from implanted tumors and the survival of irradiated and explanted cells from a tumor. Lower metastasis was found for C-ions than photons when tumor cell survival was 10%. This study suggests that C-ion significantly inhibits metastatic processes much more than low-LET photons.

**Cellular and Molecular Biology Team**

Chordoma is one of the most effective targets for carbon ion particle therapy. This year, we have developed a useful chordoma cell line, U-CH1-N, out of the only chordoma cell line available in the world, and determined its radio- and chemosensitivity. Our data provide the first chronological cell survival information using cells of chordoma origin and also help explain the successful chordoma treatment by heavy ions.

HiCER, a 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, BTK2, TP53INP1) remained activated for a longer period in human cells irradiated with carbon ion particles than when irradiated 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. We have started to characterize the roles of this centrosomal protein on DNA repair mechanisms. One approach is knocking down ASPM by siRNA treatment, which demonstrated a significant increase in the radiosensitivity of several tumor cell lines. The other approach is to generate a mouse model whose Aspm orthologous gene (calp1) was conditionally disrupted. Targeting centrosomal proteins would be a promising strategy to augment the radiation effect and may also increase the treatability of certain types of tumors.

Radiation Modifier Team

The radiation modifier team has examined the following topics in 2009. 1) We measured redox potential for various natural antioxidants to develop a new radiation protector. 2) We found that the combination of X-ray and a PI3-kinase inhibitor effectively enhanced antitumor activity both in vitro and in vivo. 3) We discovered a new compound having nitroxy radical and edaravone moieties, and which has radiation protection activity in 30-day survival of mice after irradiation. 4) We measured the DRF value of 2-TDMG against X-ray-induced bone marrow death of mice and obtained a value of about 1.2 by i. p. administration of the compound (100 mg/kg) immediately after exposure. 5) Effect of radiation protectors on tumor regulation by heavy ion radiation using the mouse xenograft model was measured. 6) The effect of amifostine on tissue oxygen tension was measured by EPR oxymetry using LiNC-BuO as a probe. 7) The distribution of reactive oxygen species generated by irradiation of heavy ion beams was measured and analyzed.

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.

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Objectives

This subject has been researched by the Transcriptome Research Group consisting of 3 teams: Stem Cell Research Team, Model Organism Research Team and Gene Expression Profiling Team.

Progress of Research

1) Stem Cell Research Team and Model Organism Research Team

This year the two teams have focused on pluripotent stem cells; their final goals are to understand the effect of radiation at an individual level - not at a cellular level only - and to obtain relevant information for their therapeutic uses.

Recently, it has been demonstrated that somatic cells can be converted into pluripotent stem cell by ectopic expression of four genes, Oct3/4, Klf4, Sox2 and cMyc, designated as induced pluripotent stem (iPS) cells. The objective of this program is to understand the molecular mechanism of the conversion from somatic to stem cells. However, it has been rather difficult because these cells emerge at a low frequency, about 0.1% in the case of fibroblasts, and in a stochastic manner. Therefore, the teams attempted to directly observe the emergence of iPS cells from somatic cells. To overcome the difficulties, team members developed a new investigation system by improving a pre-existing time-lapse system that allows us to precisely investigate the generation of iPS at short intervals over 2 weeks that are needed to generate iPS cells from mouse fibroblasts, and succeeded indirectly observing the conversion process of somatic cells into stem cells. Interestingly, it was revealed that the onset of the cell lineage conversion already initiated within 48 hours just after the defined gene infection in most of the iPS cell generations, i.e. 85.7% (Figure 3-7). Furthermore, no morphological asymmetric cell division was observed during the conversion process from ancestral somatic cells into iPS cells. Namely, ancestral fibroblast cells gradually transformed into stem cells after several symmetric cell divisions (Figure 3-8). Thus these results provide a critical new insight during the first three days of iPS cell generation that has thus far been completely unknown. In addition, another contribution to the iPS field has been made: genome integration-free iPS cells without oncogene, c-Myc, transduction have been successfully generated.

2) Gene Expression Profiling team

This team has developed an ideal transcriptome analysis procedure called High coverage gene expression profiling (HiCEP) that is based on a principle different from hybridization-based methods.

This year this team attempted to improve the HiCEP method to allow it to analyze a small amount of starting material. At the beginning of HiCEP development, approximately 1 μg of polyA RNA was needed for the analysis; however, subsequent improvement has allowed us to perform the analysis with a total RNA amount of 0.1 μg. This year team members successfully developed a new protocol using less than 100 μg of total RNA, corresponding to less than 10 cells.

Meanwhile, they developed new systems for efficient HiCEP analysis: firstly a high throughput machine for HiCEP reaction that enables more than 15,000 samples per year to be analyzed. This equipment, termed HiCPer, will be released this year. A precision PCR (polymerase chain reaction) machine was also developed and released, because the HiCEP reaction requires an extremely high level of temperature control. The difference in temperature among 96 wells can be controlled by less than 0.2°C. In addition, a HiCEP reaction kit was developed, for 1 μg of starting material, which allows even those who do not have expertise in molecular biology to perform HiCEP analysis easily.