（Annual Report）1996-1997

1.The Effect of Target Fragmentation on the Autoactivity Induced by Heavy Ion Therapy

Takehiro Tomitani, Mitsutaka Kanazawa, Kyosan Yoshikawa, Yasuhiro Wada*, Ikuhiro Shinoda*

(* Siemens-Asahi Medical Technology Inc.)

keywords: positron, target fragmentation, heavy ion therapy

In 12C heavy ion therapy, some 12C heavy ion beams transform themselves to 11C through fragmentation reaction with target material. Hence this phenomenon is referred t as autoactivation of the projectiles. Likewise some target nuclei also become radioactive through fragmentation reaction of the target nuclei, which is referred to as target fragmentation hereafter. 11C autoactivity is useful for checking heavy ion treatment, since 11C is a positron emitter and the distribution can be measured by positron emission tomography(PET). After physical experiments with phantoms and experiments on animals, the technique was applied to patients. Each trial underwent inspection by the comittee on clinical trials. In the physical and animal studies, we observed 11C activity peak attributed to the projectile fragentation of 12C promary beams and 11C activity due to target fragmentation that distributes uniformly and forms a pedestal. The calculations based on the semi-empirical fragmentation cross-section formula(L. Sihver, T. Kanai *et al*: Phys. Rev. 47(3):1225-1236, 1993) indicated that the 11C activity induced by target fragmentation distributes almost uniformly over the entrance region to the and-points of projectiles. In these cases, the media were assumed uniform. On the other hand, 11C activity induced from projectile fragmentatio concentrates mainly near the end-points of the promary beams and is reasonably higher than that due to target fragmentation.

11C activity in the patient undergone 12C heavy ion therapy was measured. (The images are not shown here as they are best seen by using color display.) The patient had a metastatic brain tumor induced from lung cancer. In the PET image, 11C accumulates around the target region. In addition, an unexpected accumulation of activity is observed in the region near the head surface. The purpose of this report is to explore the source of the latter activity. Activity induced in the target region may be partly washed away by blood flow and recirculated to the surface of the head. However, the activity in the blood would be faurly low and distributed almost uniformly over the whole body, so that this possibility can be ruled out. Another possibiity is positron emitters induced through target fragmentation reaction. Typical atomic constituents of various organs are shown in Fig. 1. Possible positron emitters are 11C(t1/2＝20.39 min.), 38K(t1/2＝7.636 min.). Potassium isotopes can be exclided because of relatively short half-lives. 38K may be induced from 40Ca. The density of the bone(P ～ 1.82) is almost twice as high as densities of other organs(P～1) and the atomic fraction of C is higher thatn in other organs except adipose tissure. To

explore these two possibilities, we performed three experiments. In the first experiment, a polyethylene cylinder (15 cm diameter, 10 cm height) was attached in front of the cylinder. In the third experiment, a stack of three lenses made of CaF2 (3 cm diameter, 1.5 cm thick in total) was attached in front of the cylinder. Fluorine in CaF2 might induce 18F, but its half-life (109.77 min.) is longer than that of 11C, so that 18F can be distinguished from 11C activity by time-activity analysis. The results are shown in Fig. 2, from which we conclude that the activity in the skull region can be attributed to 11C induced from target fragmentation of 12C in the skull and not to 38K from 40Ca.

This result indicates that fat tissue will also contribute to the generation of 11C through target fragmentation, since C fraction in fat is about 1.5 times higher than that of bone, even though its density (P $\sim$ 0.92) is 0.5 of that of bone. In fact, we observed high activity accumulation near the surface in the PET image taken of a patient with a liver tumor, in which rib bone may be another source.

Publications:

Yoshikawa, K., Tomitani, T., Kanazawa, M., Wada, Y. Kanai, T., Imai, Y., Suhara, T., Kato, H., Koga, M., Kandatsu, S., Yoshioka, H. and Tsuji H.,J. Nucl. Med. Technol. 24, 167-168, 1996.

Tomiaki, T., Yoshikawa, K., Kanazawa, M. et al.: in *Advances in Hadrontherapy*, U. Amaidi, B. Larsson and Y. Lemoigne, editors, Elsevier, Amsterdam, pp.339-345, 1997.

Figure captions

Fig. 1. Atomic constituents of human organs. Data were cited from ICRU report 37, pp.27-29.

Fig. 2. Positron emitter distribution induced from 12C heavy ion beams. Left: Psitron emitter distribution in the polyethylene cylinder; middle: that in the polyethylene cylinder with a graphite slab in the upstream positron; right: that in the polyethylene cylinder with CaF2 lenses in the upstream positron.

2. Effects of Nuclear Fragmentation Reactions at Relativistic Energies

Susumu Kinpara

Keywords: nuclear fragmentation reaction, cross section, 12C

One interesting dynamical phenomenon in physics is heavy ion nuclear reactions, which yield mass number distribution. Various experimental data have been accumulated, but now, it is necessary to look at the reactions systematically through a theoretical investigation in order to comprehend nuclear interactions and structure of atomic nuclei.

In particular, recent developments of the nuclear many-body problem allow nuclear structure to be described using the meson exchange interaction expressed in terms of the meson degrees of freedom explicitly. Such results encourage study of nuclear interactions by means of a microscopic treatment.

Knowledge of reaction processes considerably affects studies in fields other than nuclear physics. For example, the use of heavy ions has become feasible recently in the field of radiotherapy treatment for cancer diseases utilizing ionization by the particle beam. It is necessary to study the nuclear reaction processes in order to evaluate microscopically the energy loss in matter or the biological effects on cells.

When a radiation beam passes through matter, the kinetic energy of the radiation is usually lost by transferring the energy to the electrons in the atom. In addition to this scattering process, the kinetic energy of the heavy ion also possibly transfers to the matter through nuclear fragmentation reactions.

As another example, atomic nuclei are known to propagate in a galaxy with high kinetic energies as cosmic rays. Knowledge of the cross section of the spallation is experimentally and theoretically important for understanding the creation of elements from the beginning of the universe to the present.

In this work, fragmentation reaction cross sections at relativistic energies are investigated using phenomenological approach. Equations are obtained by a geometrical treatment in the framework of classical dynamics, in which the impact parameters is introduced. A numerical solution is obtained for the 56Fe + 12C system at 600 MeV/nucleon incident energy and shown in Fig. 3. The predicted mass number distribution of fragmentation reproduces well the experimental data. The coulomb energy term in the mass formula affects the nuclide dependence of cross sections in a favorable direction.

Publication: Kinpara, S.:J. Phys. Soc. Jpn, 66 522-524, 1997.

Fig.3 Mass yield cross sections σ(units, millibarn (mb)) fr the mass number A of the fragments. The data are connected by guidelines. The experimental data are from the literature.

3.Measurements of Nuclear Tracks on CR-39 with Atomic Force Microscopy

Mikio Yamamoto, Nakahiro Yasuda, Minori Yamagishi1, Youji Kaizuka2, Mieko Kurano, Tatuaki Kanai, Akira Furukawa, Nobuhito Ishigure, Masaharu Nakazawa2,Hiroyuki Takahashi2, Tadayoshi Doke3 and Koichi Ogura4 (1Toho Univ., 2Univ.of Tokyo, 3Nihon Univ., 4Waseda Univ.)

Keywords: track detector, CR-39, etch pit, heavy ion cancer treatment, atomic force microscope

Recently, some demonstrations of an atomic force microscope (AFM) have been made taking images of the track etch pits of the solid state detector (SSNTD), but reports on quantitative analysis of track etch pits using the AFM technique are limited.Object sizes for AFM measurements are about 1/100 - 1/1000 of those for OPT measurements. Therefore, AFM is a very useful tool to study the track formation and etching mechanism of the track detector. This method makes it possible to observe high density etch pits produced by heavy ions of more than 107 ions/cm2. It is applicable to in vivo measurements of LET and has been used to measure dose for carbon ion cancer treatments at NIRS, which use an ion density of 106 ions/cm2 (Table 1).The feasibility of applying AFM to the quantitative analysis of minute etch pits on CR-39 was studied in comparison with observations using an optical microscope (OPT).

The CR-39 detectors were irradiated by 490 MeV/u silicon ions and 290 MeV/u carbon ions at NIRS-HIMAC with a density of about 107 ions/cm2, each. The chemical etching was carried out in a solution of 7N NaOH at 70℃, using a water bath incubator at a constant temperature. The etching time was varied from 10 to 900 minutes. Etched silicon and carbon tracks with diameters ranging from 0.1-2μm were measured with the AFM and 2-10μm with the OPT. Some samples were observed using both AFM and OPT. The diameter (D) of etch pits as a funtion of etching time for silicon and carbon trradiated samples is shown in Fig. 4. In order to chack the cnsistency between the two techniques, some samples were measured by both OPT and AFM. The data obtained by the two methods are connected smoothly without any inconsistency. While the track diameter is almost constant for the layer of 0.5-9.0 μm, it decreases a little in the surface layer under 0.5μm. The result of this study will be useful for understanding the track formation mechanism of SSTD. Moreover this AFM technique makes it possible to analyze the high track density of 106 - 108 tracks/cm2.

Table.1 Comparison of measuring methods.

	OPT	**AFM**	**Ref. Cancer treatment**
Density (ions/cm2)	104	108	106
Etching (hours)	24	0.1	

Fig.4. Variations of diameter of etch pit obtained by AFM (white points) and OPT (black points) are shown as a function of etching time.

Publication:

M. Yamamoto, N. Yasuda, et al., Radiation Measurements, 28(1997)227.

4．Measurements of Bulk Etch Rate of CR-39 with Atomic Force Microscopy

Nakahiro Yasuda, Mikio Yamamoto, Nobuyuki Miyahara, Nobuhito Ishigure, Tatuaki Kanai and Koichi Ogura* (*Nihon Univ.)

Keywords: atomic force microscope, track detector, CR-39, bulk etch rate

For the atomic force microscope (AFM) observation of minute tracks (≦1μm) in CR=39 track detectors, an accurate method for the measurement of the extremely small amount of bulk etch is required. A simple method for the direct measurements of the bulk etch using AFM was developed.

The bulk etch, B, is the amount of material removed from each surface of a solid state track detector (SSTD) during its chemical etching. The B is an important parameter for determining the track sensitivity of SSTD. The traditional methods for measuring B are through the measurement of 1) the change in the detector thickness, 2) the mass change of the detector, 3) the diameter of high-LET particle tracks such as fission tracks. In the case of the short etching time required for the AFM observations, the thickness and the mass changes of the etched detector are extremely small. Therefore, the methods 1) and 2) are not applicable to determine B for these samples. Method 3) was chosen for comparison to the developed method.The small CR-39 samples (1x1 cm2) were irradiated to 252Cf fission fragments in air. After the irradiation, a part of the CR-39 surface exposed to fission fragments was masked with epoxy resin adhesive. The partially masked samples were etched in 7N NaOH solution at 70℃ using a water bath incubator. The etching time was varied from 3 to 60 minutes. The mask was easily peeled off from the CR-39 surface of CR-39 after etching. The surface of the detector were imaged directly by AFM.

Since the part of the surface masked by epoxy resin was not etched, a step appeared on the CR-39 surface as shown in the AFM image of Fig.5. Then the amount of bulk etch, B, of the detector was directly measured as a level difference of this step.

These results were compared with those by the conventional measuring method described as the method 3) in which, diameters of fission tracks on the etched surfaces of the same samples were also measured by AFM. It is well known that the average radius of fission tracks corresponds to the amount of B of the etched sample. The comparison for the two methods is shown in Fig.6. The measured amounts of B for CR- 39 are in good agreement between each other within the statistical errors.

By using AFM, it is expected that precise analysis for the minute tracks in CR-39 can be made to get the etching properties, the etch induction time and the track sensitivities for various ions including low velocity ions. The results of those studies will be useful for understanding the track formation mechanism of SSTD.

Fig. 5. An AFM image (153x153 μm2) of masked-atched surface. The CR-39 was etched in 7N NaOH at 70℃ for 10 minutes. This image is displayed as a viewer 30℃above the surface would see it. The level difference shows the amount of bulk etch (B).

Fig. 6. The radius of etch pits vs. amount of bulk etch.

Publications:

M. Yamamoto, N. Yasuda, et al., Radiation Measurements, 28(1997)227.

5．An Experiment on Remote Action against Man in Sensory-Shielding Condition (Part II)

Mikio Yamamoto, Masahiko Hirasawa, Hideyuki Kokubo,
Kimiko Kawano1, Tsuyoshi Hirata2, Tomoko Kokado, Hideaki Sakaida,
Nakahiro Yasuda, Akira Furukawa and Nobuo Fukuda (1 Nippon Medical School, 2 NEC Corporation)

Keywords: qigong, tohate, sense shielding, suggestion, extrasensory, remote action, EEG

"Tohate" is a term from traditional Japanese martial arts. When tohate is performed, the receiver feels a sensory shock and steps back rapidly when a master of the martial arts (the sender) emits "qi" to the receiver without any touching. Tohate is seen as a signal translation by qi. However, we do not have complete theories explaining tohate or qi. In our earlier experimental studies, we reported that the phenomenon of tohate when performed by a qigong master is not caused only by the master's suggestion.This report examines our earlier results by new experiments which were done under randomized and double blind conditions.

The sender (qigong master) and the receiver (his pupil) were separated in two rooms of a sensory-shielded building, with the receiver on the 1st floor and the sender on the 4th. The sender performed one "qi-emission" action during each 80 sec trial at a random time indicated by the experimenter. When the sender performed a remote action, the sender's qi-emitting motion time and the receiver's response motion time (start of the step back) were recorded. The coincidence frequency within ±5.5 sec was 16 (expected value was 7.88) for the 49 trials. It is statistically significant and the p-value is 0.0008 (post hoc analysis).

This suggests that there is an unknown communication mechanism between the sender and receiver. The coincidence frequency is very high in the range from -5.5 to +5.5 sec. It is especially high in the range from -1 to +1 sec in our earlier experiments. However, there was no remarkable ±1 sec peak in the new experiments. The reason for the difference between the previous and new results may be due to the change of experimental conditions.

In more experiments using the same sender and receiver, electroencephalograms (EEGs) of the receiver were recorded. The qi-emission was performed at a random time selected within a minute period by the experimenter. For 57 trials there is a statistically significant difference between the emitting and non-emitting times in the alpha wave mean amplitudes of the EEGs for the right frontal part of the brain for the receiver. This suggests that extrasensory information transfer may take place and that it may be related to the right frontal part of the brain.

Publications:

1) Yamamoto, M., Hirasawa, M., Kawano, K., Yasuda, N. and Furukawa, A.: J. Intl. Soc. Life Info. Sci., 14, 97-101, 1996.
2) Yamamoto, M., Hirasawa, M., Kawano, K., Kokubo, H., Kokado, T., Hirata, T., Yasuda, N., Furukawa, A. and Fukuda, N.: J. Intl. Soc. Life Info. Sci., 14, 228-248, 1996.

6. An Experiment on Extrasensory Information Transfer with Electroencephalogram Measurements

Masahiko Hirasawa, Mikio Yamamoto, Kimiko Kawano*, Akira Furukawa and Nakahiro Yasuda (*Nippon Medical School)

Keywords: subconscious, extrasensory, information transfer, EEG, alpha wave amplitude, right frontal region

A sender and a receiver were located in two separate, sensory-shielded rooms. Their electroencephalograms (EEGs) were measured at 12 points (Fp1, Fp2, F7, F8, C3, C4, T5, T6, O1, O2, Fz, Pz) with monopolar leads both while sending and not sending extrasensory information.
To transmit extrasensory information, an experimenter showed the sender a card for a few seconds that was randomly selected from forty non-picture cards. The sender then concentrated on that card with the eyes closed for one minute. The receiver was previously informed that the information to be transmitted consisted of one of forty non- picture cards. During the non-sending period, the sender thought nothing for one minute with the eyes closed.
The sending and non-sending of extrasensory information were each carried out for one minute in a two-minute period. The experimenter randomly decided and notified the sender whether the first or second half were to be used for sending. The receiver was only informed of the start time and one and two minutes later with phoneticsigns and was not informed which half was used for sending. The receiver guessed both the sending information and sending time period, with the eyes closed during the two-minute trial. This trial was repeated 20 times.
The experiment was carried out for two pairs of subjects (A and B) using two rooms separated by a corridor on the fifth floor of the First Research Building at NIRS on the 11th of October, 1995.
Conscious recognition by the receivers in guessing the time when information was sent was not significant at the 5% level of significance (one-tailed) for either pairs. Thus, extrasensory transfer of information between the subjects' consciousness was not demonstrated. On the other hand, there was a difference in the α wave mean amplitudes calculated from Pair A receiver's EEGs between the sending and non-sending tome zones that was judged significant at the 5 % level of significance (one-tailed) at one point (O2) in the period 20 to 25 seconds after the start of the sending and non-sending times.
Likewise, there was a difference for Pair B at one point (Pz) in the 20 nto 25 second period from the start, at three points (Fp2, F7, F8) in the 30 to 35 second period and at one point (O2) in the 50 to 55 second period. These results suggest that extrasensory information is transferred between the subjects' subconscious.
The reactions for this extrasensory transfer of information occur first in the occipital to parietal regions and next in the right frontal regions of the receivers. The occipital is a visual region, while the frontal region is concerned with integration. Since the extrasensory transfer of visual onformation was attempted in this experiment, one hypotesis is that the visual regions react first and compose visual information. This is followed by the integration regions reacting to the information. However, the α wave differences were 10

seconds or more between the reactions in the visual and frontal regions. Thus, these reactions are different from those of ordinary conscious recognition.

Comparisons of the significant changes in the receivers and senders of the differences of the α wave mean amplitudes obtained from the EEGs between the sending and non- sending times indicate that there is no correlation between the senders and receivers.

This shows that the extrasensory transfer of information in the subject's subconscious suggested in this experiment is not completed instantaneously.

Publication:

Hirasawa, M., Yamamoto, M., Kawano, K., Furukawa, A., and Yasuda, N.: J. Int. Soc. Life Info. Sci., 14: 185-195, 1996.

7．Dosimetry of Particle Beams with Different Walled Ionization Chambers

Takeshi Hiraoka, Kaname Omata, Akifumi Fukumura and Mitsue Takeshita

Keywords: ionization chamber, absorbed dose, wall material, absorbed dose comparison

In Japan, absorbed dose for radiotherapy beams must be determined according to ｢the protocol for the dosimetry of high-energy photon and electron beams in radiotherapy｣ which is published by the Japanese Association of Radiological Physicists (JARP). The protocol requires to use of the JARP-type ionization chamber which is basically a Farmer ionization chamber made of PMMA for both the wall and build-up cap. Several types of Farmer ionization chambers are commercially available. Then, for this investigation we determined the absorbed dose to water for heavy ion beams were by using several types of Farmer ionization chambers with different wall and build-up cap materials.

We prepared five different wall and build-up cap combinations PMMA-PMMA, Nylon- PMMA, Carbon-Delrin, A-150-Lucentine and C-552-Polystyrene. All ionization chambers had the same geometrical conditions with very small differences of length and diameter of the outer and central electrodes. The chamber readings were calibrated by 60Co gamma rays whose intensity had been calibrated by a chamber traceable to the national standard.

Absorbed dose measurements were carried out for 290 MeV/u carbon ion beams from HIMAC(Heavy Ion Medical Accelerator in Chiba). Measurements were also made for 70 MeV proton beams from NIRS AVF cyclotron. Irradiations were made at the entrance plateau for a mono-energetic beam and at the center of the Spread Out Bragg Peak(SOBP). Irradiations were carried out in air without a build-up cap.

Ionization charges were measured by an electrometer, Keithley model 6517, which has built into a 1000V high voltage source. We used 500V as the polarizing voltage for the measurement of heavy ion and proton beams, except for the chamber made by A-150 wall which used 400 V.

One of the most important problems in the experiment of dose comparison is the stability of beam monitor. In the HIMAC irradiation facilities, three beam monitor system are installed, two ionization chamber monitors and a secondary emission monitor. In order to check the stability of the beam monitor, measurements were repeated regularly using a reference ionization chamber. Then, the stability was estimated within 0.2% for both heavy ion and proton beams.

Before the measurements, we examined the physical constants appearance in the dose evaluation formulae, especially for the 60Co calibration beam. The values were taken form the data of Andreo and the values used for the IAEA protocol.

For all chamber irradiations, measurements were made for both positive and negative polarities. The polarity effect for all chambers was less than 1%, and the largest value was 0.61%. So the effect was very small for the chambers investigated, especially for the charged particle beams. Actually, absorbed dose was calculated using the average value for both polarities.

The ionization chamber readings were converted to absorbed dose to water according to the JARP protocol. The dose was expressed in Gy for appropriate monitor counts. No corrections were made for the

very small differences in ionization chamber geometry.

The range of variation of the absorbed dose estimated for all chambers was within 0.8% for both particle beams. The maximum standard deviation was 0.45% for SOBP at 117mm.

We concluded that the absorbed dose to water for heavy ion beams, which was determined with several Farmer-type ionization chambers, showed good agreement, within 0.8% according to the JARP protocol. The small difference of the absorbed doses may depend on inaccuracy of physical constants used for the chamber calibration.

8．Small-scale Dosimetry Comparison for Therapeutic Carbon Beam at HIMAC

Akifumi Fukumura, Takeshi Hiraoka, Kaname Omata, Mitsue Takeshita, Kiyomitsu Kawachi, Tatsuaki Kanai, Naruhito Matsufuji, Hiromi Tomura, Yasuyuki Futami and Guenther Hartmann* (*DKFZ, Germany)

Keywords: absorbed dose, carbon beam, dosimetry intercomparison, HIMAC

The traceability for the absorbed dose of proton and heavier ion beams has not been established by national standard laboratories so far. Therefore it is important for heavy ion therapy facilities to compare the absorbed dose evaluated by their own dosimetry system with others and to establish a standardized base for the dosimetry. The dosimetry comaparison experimant between Japanese and German heavy-ion therapy dacilities was carried out with the 290 MeV/u carbon beam at HIMAC in December 1996, following the comparison held at GSI in March 1996(G. Hartmann et al.,*Advances in Hadrontherapy*, 346-350, 1997).

The dosimeters which each dacility used were Farmer-type air-filled ionization chambers manufactured by PTW. Each chamber was installed behind the binary filters made of polymethylmethacrylate and irradiated with the carbon beam for which quantity had been preset by the beam monitor. The measurement of the carbon beam was carried out under three conditions as follows:

at 0 cm depth for the monoenergetic 290 MeV/u carbon beam;

at the depth equivalent to 12 cm in water for the monoenergetic 290 MeV/u carbon beam; and

at the depth equivalent to 12.1 cm in water for the carbon beam which has the range of about 15 cm and the Bragg Peak spread out to a 6 cm width, both in water.

Each facility individually evaluiated the absorbed dose in water from electrical charge collected by its own chamber, according to the dosimetry protocol adopted by the facility. The protocol of the Japanese group is based on the European proton dosimetry protocol(S. Vynckier, et al., Radioth. Oncol., 20, 53, 1991;S. Vynckier, et al., Radioth.

Oncol., 32, 174, 1994)and uses the stopping power ratio of water-to-air for the carbon beam(T. Hiraoka and H. Bichsel, Jpn. J. Med. Phys 15-2, 91, 1995). On the other hand, the German group adapts the newest ICRU report for clinical proton dosimetry without modifications. The difference between the two proton dosimetry protocols is described elsewhere(J. Medin, et al., Phys. Med. Biol. 40, 1161, 1995).

Under the first condition, fluence measurements were also performed with CR-39 track detectors, which were processed independently at both institutes. In addition, a plastic scintillator was used for fluence measurements. The measured fluence was multiplied by the stopping power of the 290 MeV/u carbon beam t evaluate the absorbed dose in water.

Table 2 summarizes the results of this dosimetry comparison. The values in each condition are normalized to the mean value obtained with the ionization chambers. In spite of different dosimetry protocols, the values obtained by the ionization chambers are in very good agreement within experimantal errors for all conditions.Therefore, this comparison establiches a relatively standardized base between Japanese and

German heavy-ion facilities for the ionization chamber dosimetry of the carbon beam. The values obtained by fluence methods are, however, lower than those using ionization chambers.This is partly because the fluence measurements miss the contribution of the low LET particles such as fragments or secondary electrons. On the other hand, both groups substitute the differencial W-value of the low-energy proton beam, because of the lack of data on the differencial W-value for a high energy carbon beam. If the differencial W- value for the high energy carbon beam is lower or close to the W-value for an electron beam, the doses obtained with the ionization chamber would be overestimated. To establish an absolutely standardized base with high accuracy, dosimetry with a water calorimater is also required.

Table 2. Results of dosimetry comparison for carbon beam at HIMAC

Institute	**Method**	**Ratio of evaluated dose to mean value obtaned by ionization chambers**		
		Condition 1	condition 2	Condition 3
NIRS	Ion. chamber	0.996±0.001	0.999±0.002	1.000±0.001
DKFS	Ion. chamber	1.004±0.008	1.001±0.002	1.000±0.002
NIRS	CR-39	0.945±0.023		
DKFS	CR-39	0.908±0.027		
NIRS	Scintillator	0.942±0.037		

PHYSICS

9.Estimation of the Exposure and a Risk-BEnefit Analysis for a CT System Designed for a Lung Cancer Mass Screening Unit

Kanae Nishizawa, Kazuo Iwai1, Toru Matsumoto, Kunio sakashita, Takeshi A. Iinuma2, Yukio Tadaaki Miyamoto, Akimitsu Shimura3 and Hiroshi Takagi4

(1 Nihon University, 2 Saitama Institute of Technology, 3 Chiba Anti-Tuberculosis Association, 4 Hitachi Medical Corporation)

Keywords: LSCT, medical exposure, risk-benefit analysis

An integrated mobile lung cancer screening system consisting of a compact CT and a vehicle, called a lung cancer screening CT unit (LSCT) was developed. Owing to the high performance of the CT, we expected that the spiral scan system would be applicable to mass screening examinations because of its higher chances of detecting lung cancer at its earlier stages, thus resulting in better treatment scores. However, the ICRP has indicated inits publocation that there is considerable room for reducing doses in diagnostic radiology. Since the majority of the subjects undergoing mass screening examinations are healthy, the effect of radiation dose during each examination must be investigated seriously. Thus the primary objective for the LSCT development was reducing the radiation exposure dose. Another important issue is the compromise between the risk and benefit of mass screening examinations.With this in mind we measured the exposure doses and performed risk-benefit analysis based on the measured dose.
The organ or tissue doses were determined using a Rand phantom and two kinds of thermoluminescence dosemeters (TLD) under exposure conditions identical to those of routine chest CT examinations.
The resulting organ or tissue dose measurements taken during spiral scanning and the scan for positioning are shown in Table 3. The effective doses due to LSCT examination were calculated by the ICRP60 method on the assumption that the X-ray radiation weighting factors is unity. The results are included in Table 3.
The doses used in the risk calculation were 2.65 mSv for the mean bone marrow dose,
8.71 mSv for the mean lung dose and 9.42 mSv for the breast dose.The rates of cancer incidence cited from the UNSEAR '88 Report were 5.9 x 10-3 Sv-1 for RLung, 4.3 x 10-3 Sv-1 for RBreast and 9.3 x 10-3 Sv-1 for RBonemarrow. Because the doses from the CT scans could be assumed to be low doses with low dose rates, the value of 2 was adopted for dose and dose rate effective factor (DDREF) following the ICRP60. The risk was estimated based on the additive risk prediction model.
The results may be summarized as follws. 1) The effective dose due to LSCT chest examination was 3.5mSv and the surface dose was 7.6mGy.2) These doses were about 1/2 to 1/3 less than average doses from conventional spiral CT examination for the same area of exposure. 3) The risk-benefit analysis for a yearly examination basis revealed that
the benefit outweighs the risk at the age of 40 in the case of men and at the age of 45 n the case of womem.

We are now considering the basic concept of the ICRP which is that doses have to be as low as reasonably possible and are undertaking further efforts to reduce exposure doses without loss of image quality.

Table 3. Organ or tissue doses in mGy for LSCT chest examination and effective dose in mSv. The spiral scan was performed using a tube voltage of 120kV, a tube current of 50mA, a slice thickness of 10mm, a tube rotation speed of 2s and a table speed of 10mm/s for the lung cancer screening conditions.

Organ or Tissue		**Scan for positioning Mean±SD**	**Spiral Scan Mean±SD**
Gonads	Male	0.00±0.01	0.00±0.02
	Female	0.01±0.03	0.04±0.11
Red bone marrow		0.37±0.19	2.65±0.66
Colon		0.08±0.09	0.41±0.10
Lung		1.02±0.32	8.71±1.48
Stomach		0.45±0.21	4.84±1.10
Bladder		0.01±0.03	0.04±0.10
Breast		0.54±0.23	9.42±1.54
Liver		0.48±0.22	5.37±1.16
Oesophagus		0.94±0.31	8.37±1.45
Thyroid		0.03±0.06	0.36±0.30
Bone surface		0.83±0.29	6.38±1.26
Remainder(mean)		0.44±0.21	4.16±1.02
Effective Dose (mSv)	Male	0.36±0.19	3.46±0.93
	Female	0.36±0.19	3.45±0.93

SD: Standard Deviation

SD: Standard Deviation

１０．A Prescribed Diffusion Model to Estimate G-value of Fricke Dosimeter Irradiated by Photons of 100 eV-10MeV

Hiroshi Yamaguchi

Key words: dosoimetry, chemical dosimetry, Fricke dosimeter, theory, photon, synchrotron radiation

Measurements of G-value of a Fricke dosimeter have been elaborated for low energy photons, 1.5 keV(Freyer *et al*. 1989), 8.9 and 13.6 keV(Hoshi *et al*. 1992) and 1.8-10 keV(Watanabe *et al*. 1995). Those values are bases for dosimetry of biological samples in solution irradiated by synchrotron radiations, because no recommendation has been made for energies less than 5 KeV by the ICRU 17(1970). Furthermore theoretical studies have not been successful in explaining G-values (Magee and Chatterjee 1980, Yamaguchi 1987, 1989). There may still be a problem in the way track structure of electrons is connected to the yields of chemical products. This paper revises the author's previous calculation (Yamaguchi 1989) and aims at explaining the experimental data and studying underlying mechanisms of the problem. Analytical methods have dealt with radiolysis of neutral water and the Fricke G-values were estimated by a combination of yields of species using the relations of material balance. In the present work, the Fricke solution is explicitly dealt with for the first time; that is, in addition to the 12 reactions among water radiolysis species and dissolved oxygen, the following three reactions are considered.

x1010(M-1s-1)

(1) $OH + Fe^{2+} \rightarrow Fe^{3+} + OH^-$ 0.05

(2) $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH^-$ 0.05

(3) $HO_2 + Fe^{2+} \rightarrow Fe^{3+} + H_2O_2$ 0.12

All reaction processes are described by diffusion controlled reaction differential equations. To solve the differential equations, a spur of constant size is assumed as an entity, independent of electron energy, and that track structure is taken into account by restricted stopping power with a cutoff energy.

Numerical integration of the differential equation from 10-12s to 10-5s yields the differential G(Fe^{3+}) of electrons. When the energy spectrum of electrons from photons is known, the G-value of the photons can be estimated. The results are shown in Fig.7. The present calculation agrees well with the experimental data and is consistent in the region 5keV-150keV with the recommended values of ICRU(1970). Good agreement of the present calculation with the experiments is attributed to the restricted stopping power and the △ is the cutoff energy. The present calculation may be useful to interpolate or extrapolate the experimental data, or to estimate G(Fe^{3+}) where experimental data are inconsistent.

Publication:

Yamaguchi, H.: *Microdosimetry-ANInterdisciplinary Approach*-.D.T.Goodhead, P.O'Neill, H.G.Menzel, editors. Royal Society of Chemistry, 97-100,1997.

Figure caption Fig. 7. Frivke G-value for photons.

1 1． Status of the HIMAC Injector

Takeshi Murakami, Satoru Yamada, Atsushi Kitagawa, Masayuki Muramatsu, Koji Noda, Hirotsugu Ogawa, Yukio Sato, Eiichi Takada, Katsuto Tashiro, Jun Yoshizawa, Toshitaka Fukushima, Takanari Kimura, and Kazuhiro Ueda

Key words: linac, medical accelerator, HIMAC

A medical accelerator, HIMAC (Heavy Ion Medical Accelerator in Chiba), was constructed in order to investigate the effectiveness and extent of heavy-ion therapy. A clinical study began in June, 1994, and about 150 patients had been treated by the end of July, 1996. The clinical studies presently employ C beams with energies of 290, 350, and 400 MeV/u. Beams of C4+ from the ECR ion source are provided to the synchrotron. Precise positioning of the patients takes from 20 to 30 minutes, while beam irradiation continues for 2 minutes or less in typical cases. Irradiation corrected for respiration motion began in May, 1996. The treatment sites include the brain, head and neck, lung, liver, prostate, and uterus.
HIMAC is a facility, capable of accelerating ion beams from He to Ar up to a maximum energy of 800 MeV/u for q/A = 1/2. Since accelerators delivering heavy-ion beams with energies from 100 MeV/u to 800 MeV/u are very scarce in the world, applying HIMAC beams to basic research is strongly desired. Experiments, including wide areas such as physics, chemistry, engineering, and biology, are being carried out during the night and on weekends. In 1996, nearly 100 proposals were accepted and about 300 researchers inside and outside the institute participated in the experiments. Although the C beam is most commonly used, many kinds of beams, such as He, Ne, Si, and Ar are used in basic research. The beam time assigned for basic research in FY 1995 was 2200 hours in total. The HIMAC injector is comprised of RFQ and Alvarez linacs. There are two types of ion sources, 10 GHz ECR and PIG. A pulse-width controller, which varies the beam-pulseduration from 1 ｲs to 0.7 ms, was newly installed upstream of the RFQ linac. The purpose of the controller is (1) to eliminate unnecessary beams from the ion sources; and (2) to supply beam pulses having a different duration to the users. A new beam course, the medium-energy experiment course (MEXP), was constructed to utilize the beams from the injector (6 MeV/u).
To take full advantage of the two rings and the associated MEXP, a system realizing a time-sharing-acceleration mode (TSA) was investigated. It is possible to deliver different ion species to three user groups using the TSA. The upgrade to the new system is comprised of several steps: (1) installing the third ion source, an 18-GHz ECR ion source favorable to producing heavier ions such as Fe; (2) replacing all of the DC-operated magnets with pulse-operated magnets; (3) installing non-destructive-type current monitors, not affecting any other beams during the measurement, and (4) modifying the control system, especially the man-machine interface, while inheriting the present system. The scheduling of hardware/software replacements must avoid interfering with the treatment time.

Publications:

Murakami, T., et al.: J. Nucl. Mater., 248 (1997) 360 - 368.

１２．A Respiration-Gated Beam Control System for Patient Treatment

Koji Noda, Shinichi Minohara, Mitsutaka Kanazawa, Eiichi Takada, Natsuji Araki, Masami Torikoshi, Shinji Sato, Masayuki Kumada, Hiromi Tomura, Tatsuaki Kanai, Hirotsugu Ogawa and Satoru Yamada

Key words: respiration-gated irradiation, RF-KO extraction, beam deceleration, penumbra

A respiration-gated beam control system has been developed at HIMAC in order to minimize any inevitable damage to normal tissues around a tumor when the tumor moves along with the patient's respiration during treatment. The system employs mainly a position sensitive detector to sense target movement and has rf-knockout extraction and beam deceleration as a residual beam aborting system.
It is very important to accurately detect any target movement for irradiation gated by respiration. A position-sensitive detector with an infrared light source has been chosen in order to generate the respiration signal. The choice was based on the detector reliability,stability, and easy setting. The permitting irradiation signal is generated by using the respiration signal and a threshold level, only when the respiration is in the expiratory phase.
It is essential to start and stop beam extraction promptly according to the beam "on/off" signal generated by using the permitting irradiation and the flat-top signal in the synchrotron operation-pattern. The rf-knockout method with amplitude and frequency modulation is utilized in beam extraction from the synchrotron, because the extraction method has the following advantages: 1) prompt response (within 1 ms) to start and stop beam extraction by turning the transverse rf field on and off; 2) flexible choice of the start/stop timing of extraction at the flat-top because the fields of all extraction elements are kept constant during the flat-top.
In order to reduce the effective irradiation-time for treatment, all of the accelerated beam should be extracted as soon as the "beam on" signal is generated. An irradiation period more than 10-times longer than that of the wobbling magnets is required, on the other hand, in order to obtain a uniform dose in the lateral distribution. The amplitude of the transverse rf field for rf-knockout extraction is determined so that all of the beam is extracted during about 400 ms; the flat-top period is about 1500 ms long in the 0.3 Hz operation-pattern.
The residual beam must be aborted around the ring, because the accelerated beam should not be extracted from the ring as long as a "beam off" signal is generated. Thus, as a beam-aborting system, deceleration of the residual beam from the top-energy to an injection-one is utilized in order to avoid any unwanted activation around the ring.
For a performance testing of the system, the penumbra sizes in three cases, i.e., a fixed phantom, gated irradiation to a moving phantom, and ungated irradiation to a moving phantom, were investigated by measuring the density of an exposed X-ray film attached to a phantom. The penumbra size in the fixed phantom is naturally less than those in the other cases. However, the penumbra size during gated irradiation is considerably reduced to 30% that in the ungated one.

Irradiation gated by respiration has been carried out since June, 1996, after a global test of the system (Fig.8) was completed and instructions were given to operators. The system has played an important role in clinical studies related to treatment for lung or liver cancer in which tumors moved along with respiration of the patient.

Publications:

1) Noda, K., *et al*., Nucl. Instru. and Meth. A374, 269 (1996).

2) Noda, K., *et al*., Proc. 5th EPAC, Spain, 2656 (1996).

3) Kanazawa, M., *et al*., Proc. 5th EPAC, Spain, 2653 (1996).

Fig. 8. Global test for the respiration-gated beam-control system.

(a) Operation pattern of the synchrotron.

(b) Respiration pattern.

(c) Permitting irradiation signal.

(d) Circulating-beam intensity.

(e) Spill of the extracted beam.

1 3．Development of the Gated Irradiation System Synchronized with Respiratory Motion for use in Heavy Ion Therapy

Shinichi Minohara, Tatsuaki Kanai, Kouji Noda, Mitsutaka Kanazawa, Msahiro Endo, Hiromi Tomura and Kiyomitsu Kawachi

Keywords: respiratory motion, gated irradiation, gated CT

Targets such as a lung or liver cancer move due to a patient's autonomous respiration. In the conventional method, the margin around the clinical target volume is expanded by considering the motion of the target, and the irradiating volume of normal tissue increases. To concentrate the dose on the moving target, the gated irradiating method coincident with the patient's respiratory motion is very effective. In this method, the pulsed beam is repeatedly irradiated to the target at a timing synchronized with the patient respiratory

(1) Respiratory sensor
(2) Gate signal generator
(3) RF-knockout beam extracting system
(4) Gated CT for treatment planning
(5) Positioning system using x-ray TV

The motion of the target due to respiration is detected as motion of the body surface around the chest wall or back. The signal from the respiratory sensor is like a sine wave and is called a respiration waveform. A timing signal to request beam irradiation is sent in response to the respiration waveform, which is synchronized with the motion of the target, and the charged particle beam is extracted from the synchrotron of HIMAC by using an RF-knockout extraction method. In order to get accurate treatment planning- base CT images, we use an improved CT scanner to scan in synchronization with respiratory motion. For the patient positioning, x-ray TV images of the moving target are employed.

Respiratory sensor

The respiratory sensor must be easily set, non-invasive, cause no patient discomfort, and not disturb the irradiation. Furthermore the sensor signal must have a good stability and reproducibility in every fraction. To satisfy these requirements, we developed a respiratory sensor system using a position-sensitive semiconductor detector (PSD) , which is used industrially. The sensor system includes the PSD camera and the far infrared luminance electron diode (fr-LED) . The fr-LED is placed on the patient's body around the chest wall, and the change of the fr-LED position is detected by the PSD camera. The light spot of the fr-LED is focused on the PSD through the lens system of the camera. Without the software, the PSD outputs analog signals in direct proportion to the spot position. The actual motion of organs by respiration is three-dimensional and includes their shape change. In lung and the liver cancers, however, the target mainly moves along the body axis. In our current system we assume that the target motion is two-dimensional on the A-P plane. The motion in other directions and the shape change are important to dice the irradiating margin of the target during treatment planing. The amplitude of the motion is less during the expiratory phase than the respiratory phase.

Therefore the irradiation should be done in the expiratory phase.

Gate signal generator

To irradiate the target in the expiratory phase, we generate a gate signal to request the beam irradiation at this timing by applying the threshold level to the respiration waveform. This method is very simple and easy to realize with only a hardware device. The level of the threshold is set manually and monitored with the respiration waveform. On the other hand, the synchrotron of HIMAC is always operated with a regular pattern and it is possible to extract the pulsed beam during the flat-top. When the gate timing corresponds to the flat-top in the synchrotron, the beam is extracted by the RF-knockout method.

Gated CT for treatment planning

To get accurate treatment planning-base X-ray CT images, we improved the CT scanner so it can scan, synchronized with the respiration waveform. The start of scanning is triggered by the gate signal which is the same signal as the gate for beam irradiation.

The scanning time for 1 slice is 1 second. The CT table moves to the next slice position after the scanning, and the procedure is repeated. It should be kept in mind that the gated CT images include the effect of the motion for 1 second during the scanning, but the timing of the motion is the same for every CT slice.

Positioning system using x-ray TV

The moving organs on x-ray TV images at right angles to each other are monitored and read by the positioning computer. Positioning images, DRR images which are reconstructed during planning or simulation images, are displayed on the monitor screen, and if necessary we can superimpose an outline of the patient collimator, target and landmarks on these images. After referring to the anatomical bone structure and/or implanted markers on these images, we set the patient. The outline of the patient collimator, which is made of wire, is set on the beam port, and is projected on the x-ray TV image from the beam direction. Finally we verify if the moving target is kept within the collimator's outline during the gated timing, and then we start the gated irradiation. If the respiration waveform becomes unstable, we can manually stop the gated signal for a moment, and restart the irradiation after breathing is stabilized. And if necessary, we can re-position the patient and re-set the threshold level again.

Patient treatment

In February 1996, we started patient treatments with gated irradiation of a carbon beam at HIMAC. As of February 1997 we had treated 36 patients by gated irradiation;13 patients had liver cancer, 21 patients had lung cancer and 2 patients had mediastinal. At present we are using the gated irradiation routinely.

Fig. 9 Irradiation system coincident with a patient's respiratory motion for use in HIMAC.

１４．Distribution of Fragment Particles in Heavy Ion Therapeutic Beam

Naruhiro Matsufuji, Hiromi Tomura, Yasuyuki Futami, Akifumi Fukumura, Akio Higashi, Toshiyuki Kohno and Tatsuaki Kanai

Keywords: fragment, spallation, counter telescope, beam quality, particle identification

It is well known that the high-energetic heavy charged particles used for radiotherapy are broken into fragments in a patient's body by a spallation reaction. Biological effectiveness of these fragments is considered to be expressed as a function of the atomic number and the energy. However, information on each element's ratio and its energy spectrum in the beam, i.e. 'beam quality' has not been fully installed in our current treatment planning system because of the lack of a reliable theoretical model and experimental data. In this study, fluence of these fragments was measured to obtain the information on beam quality experimentally. Results were compared with calculations by the simulation code 'hibrac' produced by Sihver et al.

Experiments were carried out at the port for biological experiments (BIO port) at HIMAC. Beam delivery devices have positioned in the same way as those of the horizontal therapeutic port. Incident beam was broadened in the lateral direction to about 10 cm in diameter with a flatness better than +/-2 % by using a pair of wobbler magnets and a scatterer. Measurements were done for a carbon beam (290 MeV/nucleon) which has been used for clinical trials. Helium (150 MeV/nucleon) and neon (400 MeV/nucleon) beams were measured for comparison. As for target material, we selected PMMA (polymethyl methacrylrate, Lucite) as a substitute for body tissue. A stack of PMMA plates had already been installed as part of a beam delivery device (binary filter) in the BIO port.

The detector system was based on the △E-E counter telescope method. An NE102A plastic scintillator (1 mm thick) was located at the most upstream position of the port to count the number of incident particles. To identify fragment particles produced in the binary filter, a combination of another NE102A plastic scintillator (5mm thick) and a BGO　scintillator (300mm thick) was used. A surface-barrier silicon detector was used to measure each particle's energy loss in the detector. Measurements were done by varying the thickness of the PMMA target.

Each group of fragment particles was clearly separated on the △E-E scatter maps from promary particle down to hydrogen, according to the difference of atomic number or mass number (Fig.10). Each band was identified by comparing reciprocally the trace of promary particles at each incident beam. Fluence of fragments was derived as a function of PMMA thickness. The results were compared with the calculations based on Sihver痴model. Generally good agreement was obtained, however, disparities were seen for light fragments, such as hydrogen or helium.

15. Development of 3D Irradiation System for Heavy Ion Radiotherapy　at HIMAC

Yasuyuki Futami, Hiromi Tomura, Naruhiro Matsufuji, Akio Higashi, Makoto Fujita* and Tatsuaki Kanai (*Accelerator Engineering Corporation)

Key words: heavy ion radiotherapy, irradiation system, conformation therapy

One of the most important goals of radiotherapy is to localize the exposuredose to the target volume. Consequently, the clinical irradiation system at HIMAC was designed based on the broad beam method to conform the exposure dose to the target volume. The uniformity of the field can be kept within ～ 1.5 % by selecting appropriate combinations of the scatterer thickness and the beam wobbling radius. A spread out Bragg peak (SOBP) is realized by inserting a ridge filter upstream from a range shifter. The ridge filter, which is made of aluminum, was designed to get a uniform survival fraction of Human Salivary Grand tumor cell in the SOBP.

In the present 2D irradiation, range modulation is done by the ridge filter creating a fixed-width SOBP. The SOBP width is determined by the thickest part of the target

volume along the beam direction, and the Bragg peak is spread out to the same width for all rays in the beam. The treatment volume is a cylinder, whose length is equal to the SOBP width. The distal envelope of the treatment volume is shaped by a compensator to conform to the distal surface of the target. Ordinarily, the longitudinal thickness of the target volume is not uniform and exposure of the normal tissue upstream from the target is unavoidable, especially for an irregular-shaped target.

To improve this situation, we developed a three-dimensional irradiation system extended　from the present 2D system. In our Broad Beam 3-Dimensional Irradiation (BB3DI) system, the treatment volume is divided into "irradiation" slices. In order to reduce their number, the Bragg peak is slightly spread out to a `minipeak' of 5 mm water equivalent width by a ridge filter, and this minipeak is stacked by changing the range with a pair of wedge absorbers for which thickness can be remotely controlled. During `sweep', any unnecessary part of the irradiation field can be cut by the multileaf collimator. To realize precise control of these devices, we improved the irradiation procedure by adding a "beam inhibit gate" which prevents irradiation during its gated period. This technique was developed for the irradiation system gated with respiratory motion, and it has been applied to clinical trials since June 1996. Correct control for the BB3DI system was realized with the inhibit gate of 250 ms width, which is enough time to cover the transition time for the wedge absorber to move to the next irradiation slice.

In order to check the performance of all devices of the BB3DI system and to evaluate the quality of its irradiation field, the two-dimensional dose distribution on a plane across the center of a 7-cm diameter ball-shaped target of PMMA (polymethyl methacrylate) was measured by sweeping a small parallel plate ionization chamber. In the experiment, the 290 MeV/n carbon beamwas used. Measured lateral dose distributions for each depth were in agreement with the planned dose distributions.

16. The Local Noise Property in a Reconstructed Image of a Uniformactivity Sphere for 3D Positron Emission Tomography

Hideo Murayama

Keywords: image reconstruction, positron emission tomography, nuclear medicine

Positron imaging has gained wide use in diagnostic nuclear medicine. In particular, three dimensional (3D) positron emission tomography (PET) is a promising technique for localization and quantification of receptor-binding or tumor-specific agents owing to its higher sensitivity than two dimensional (2D) PET. Poisson noise propagates similarly into the image space with both 2D and 3D modes in the filtered backprojection procedures. The behavior of the noise propagation in the 3D mode, however, may be much different from that in the 2D mode. This is because the 3D mode possesses non-uniqueness in its operation which is specified by one algorithm among non-equivalent valid ones, but the 2D mode does not. The reconstruction kernel in a tomograpic reconstruction procedure can be expressed by the reconstruction filter and the apodising function in both 2D and 3D modes. The reconstruction filter in the 2D mode is essentially unique and given by the ro-filter, while that in the 3D mode is not unique. This non-uniqueness comes from redundant information of the measured projections in data acquisition for the 3D mode. We suppose that in all the image space projection directions dedicated to image reconstruction are restricted within an aperture, which is a subset of the unit sphere and is defined by the maximum acceptance angle, y0. We consider a spherical region of radius Re with a uniform radionuclide uptake, located at the center of a uniform attenuating spherical object of radius Ra (Ra ≧ Re) with the attenuation coefficient, μ. Then the 3D activity distribution is given by f3(**r**) = 1 for |**r**| ≦ Re, and f3(**r**) = 0 for |**r**| ＞ Re, where r is the position vector in the 3D image space. The relative variance var{f3(**r**)} is composed of two factors, which are the relative variance var{f3(**0**)} at the center of the attenuating activity sphere, and the position-dependent factor Cra3(**r**/Re, μRa, Re/Ra, Ψ0), namely:

var {f3 (**r**) } ＝var {f3 (**0**) } ・Cra3(**r**／Re, μRa, Re／Ra, Ψ0)・

In general, the image variance does not have spherical symmetry except for Ψ0 = π/2. Left and right schemata in Fig. 11 (A) show two views of the activity sphere in a cross section of the x-z plane which illustrates the basic geometry for the maximum acceptance angle at two edge positions of the sphere, **r** = (Re, 0, 0) and **r** = (0, 0, Re), respectively. The shaded area in this figure represents possible projections passing through these positions with the maximum acceptance angle Ψ0. Discrepancy in the image variance for these two positions comes from different overlapping forms with the shaded area and the activity sphere. In the case of μ = 0 cm-1, the position-dependent factor at the edge

position |**r**| = Re on the x-y plane is (2Ψ0 + sin 2Ψ0)/(2p sin Ψ0), while its value at z = Re on the z axis is (sin Ψ0)/2. We note that these values are normalized at the center of the activity sphere, because the factor is 1 at the center. Figure 11 (B) shows the position-dependent factors at the edge positions of **r** = (Re, 0, 0) (broken curve) and **r** = (0, 0, Re) (full curve), respectively, as a function of Ψ0 in the case of μ = 0 cm-1.

Publication:

Murayama, H., Nohara, N. : Phys. Med. Biol., 42, 231-249, 1997.

Figure caption:

Fig.11 (A) Geometry of the activity sphere in a cross section of the x-z plane, in which the shaded area of the left and right views represents possible projections passing through the positions of **r** = (Re, 0, 0) and **r** = (0, 0, Re), respectively, with the maximum acceptance angle Ψ0. (B) Dependence of the position-dependent factor of the image noise, Cra3(**r**/Re, μRa= 0, Re/Ra= 1, Ψ0) on the maximum acceptance angle Ψ0 at two positions of **r** = (Re, 0, 0) (broken curve) and **r** = (0, 0, Re) (solid curve). Note that the factor is normalized at the center of the sphere.

2. CHEMISTRY

1 7 Oxidative DNA Strand Scission Induced by Copper(II)-Complexes and Ascorbic Acid

Jun-ichi Ueda, Yoshie Shimazu and Toshihiko Ozawa

Keywords: DNA strand scission, copper(II) complexes, hydroxyl radical, ascorbic acid

Oxidative DNA damage from active oxygen species such as hydroxyl radical (.OH) has been hypothesized to play a critical role in diverse biological processes including mutagenesis, carcinogenesis, radiation damage, and cancer chemotherapy. Since cellular metabolism generates superoxide (O2-) and hydrogen peroxide (H2O2), .OH may be produced by a Fenton-type mechanism in which iron reduced by superoxide decomposes H2O2. Hydroxyl radical is also considered to be generated during the reaction of iron with reducing agents in the presence of oxygen.

Copper (Cu(II)) is an important metal ion as well as iron. Cu(II) is present in chromosomes and is closely associated with DNA bases, particularly guanine. In order to understand the role of biological reductant in oxidative DNA damage by Cu(II), DNA strand scission by Cu(II) complexes and a biological reductant such as ascorbic acid was investigated. The following Cu(II) complexes were used; Cu(CyHH)2 (CyHH: cyclo(L-histidylhistidyl)), Cu(OP)2 (OP: phenanthroline), Cu(HGG) (HGG: histidylglycylglycine), and Cu(en)2 (en: ethylenediamine). Fig. 12 shows DNA strand breakage by the reactions of these Cu(II) complexes with ascorbic acid. DNA derived from plasmid pBR 322 showed 2 bands on agarose gel electrophoresis (lane 1). The foremost moving band corresponded to the native form of supercoiled circular DNA (abbreviated as SC) and the slowly moving one was the open circular form (abbreviated as OC). The treatment of DNA with ascorbic acid (lane 2), Cu(CyHH)2 (lane 3), Cu(OP)2 (lane 4), Cu(HGG) (lane 5), and Cu(en)2 (lane 6), respectively, did not change the migration pattern. The results indicated these complexes did not cause the DNA strand scission. However, the ascorbic acid plus Cu(HGG) (lane 9) or Cu(en)2 (lane 10) resulted in DNA breakage from SC to OC and the linear form (abbreviated as LIN). Finally, Cu(OP)2 plus ascorbic acid (lane 8) caused more extensive DNA strand breaks than did the other Cu(II) complexes. As a result, neither OC nor LIN were observed in the electrophoretic pattern of Fig.12.

On the other hand, it has been assumed that ascorbic acid reduces Cu(II) to Cu(I) and the resulting Cu(I) decomposes H2O2 to give .OH. In order to ascertain whether .OH was generated from the reaction of Cu(II) complexes with ascorbic acid, the ESR spin trapping experiment using DMPO as a spin trap was followed under the same conditions described in the DNA strand scission. The DMPO-OH formation was observed in the reaction of all Cu(II) complexes with ascorbic acid.

Thus, the DNA strand scission may be caused by .OH that is generated by the reactions of Cu(II) complexes with ascorbic acid. Since ascorbic acid can be present in living cells, it is reasonable to consider that Cu(II) complexes may initiate DNA damage in vivo.

Publication:

Ueda, J., Saito, N., Shimazu, Y. and Ozawa, T.: Arch. Biochem. Biophys., 333, 377-384, 1996.

Legend Fig. 12. Cleavage of supercoiled DNA by Cu(II) complexes in the presence of ascorbic acid.

Lane 1: DNA alone. Lane 2: DNA + ascorbic acid (AH) (2.5 mM). Lane 3: DNA + Cu(CyHH)2 (0.25 mM). Lane 4: DNA + Cu(OP)2 (0.25 mM). Lane 5: DNA + Cu(HGG) (0.25 mM). Lane 6: DNA + Cu(en)2 (0.25 mM). Lane 7: DNA + Cu(CyHH)2 (0.25 mM) + AH (2.5 mM). Lane 8: DNA + Cu(OP)2 (0.25 mM) + AH (2.5 mM). Lane 9: DNA + Cu(HGG) (0.25 mM) + AH (2.5 mM). Lane 10: DNA + Cu(en)2 (0.25 mM) + AH (2.5 mM). Lane 11: DNA marker. Loading 0.5 mg DNA per lane. All reactions were performed in 100 mM phosphate buffer (pH 7.4) at room temperature for 5 min.

1 8　Synthesis of Polyhydroxylated Pyrrolidines and Their Application to the Asymmetric Addition of Diethylzinc to Aldehyde

Nobuo Ikota, Hiroko Hama-Inaba, and Hidehiko Nakagawa

Keywords: polyhydroxylated pyrrolidine, diethyl zinc, D-ribonolacton, asymmetric addition, chiral ligand

Polyhydroxylated amines such as (2*S*,3*S*,4*R*)-3,4-dihydroxy-2-hydroxymethylpyrrolidine and (-)-swainsonine are potent competitive inhibitors of glycosidases and have potentialy therapeutic utility in the treatment of various diseases. In this study, we describe here　(Fig. 13) the synthesis of chiral polyhydroxylated pyrrolidine derivatives (**1**, **2**, and **3**) and their use as chiral catalysts in the asymmetric addition of diethylzinc to aldehydes. The compounds **1**,**2** and **3** were prepared starting from (2*S*,3*R*,4*S*)-3,4-isopropylidenedioxy- 2-trityloxymethyl- or 2-methoxymethyloxypyrrolidine (**4a** and **4b**) obtained from D- ribonolactone by previously reported procedures.

After *N*-*tert*-butoxycarbonylation of **4a** with di-*tert*-butyl dicarbonate and triethylamine, oxidation of the corresponding *N*-Boc derivative with RuO2 gave the lactam (**5**), which was reacted with vinylmagnesium bromide in tetrahydrofuran to afford the enone (**6**) in 37% yield. Then, the enone (**6**) was reduced with NaBH4 in the presence of CeCl3 to give the allylic alcohol (**7**). Mesylation of the allylic alcohol followed by cyclization with potassium *tert*-butoxide gave the pyrrolidine derivative (**8a**) in 74% yield from the enone (6). Ozonolysis of **8a** followed by reductive workup with NaBH4 gave an alcohol (**8b**) in 84% yield. Acidic hydrolysis of **8b** afforded the hydrochloride of **1** in 80% yield.

The compound **4b** was converted to the corresponding *N*-benzyloxycarbonylpyrrolidine by benzyl chloroformate and then treated under acidic condition (concentrated HCl- MeOH=1:20) to give a trihydroxypyrrolidine (**9**) in 76% yield. Tritylation of the primary hydroxy group in **9** (trityl chloride(1.15 equiv), triethylamine, 4-dimethylaminopyridine, dichloromethane) followed by silylation with *tert*-butyldimethylsilyl chloride in the presence of imidazole in **N**,**N**-dimethyl-formamide at 0。C afforded **10a** and **10b** (**10a**:**10b**=12.3:1) in 75% yield. Protection of the secondary hydroxy group in the major isomer (**10a**) as the methoxymethyl ether (chloromethyl methyl ether, **N**,**N**-diisopropyl- ethylamine, tetrahydrofuran(THF)) followed by desilylation of **10c** with tetrabutylammonium fluoride in THF gave **10d** in 73% yield from **10a**. Reduction of **10d** with LiAlH4 in THF gave the corresponding *N*-methylpyrrolidine derivative (*2*) in 68% yield. The sulfur derivatives, (2*S*,3*R*,4*S*)-*N*-(2-mercaptoethyl)-3,4-isopropylidenedioxy-2- trityloxy-methylpyrrolidine (**3**), was also prepared from **4a** by treatment with ethylene sulfide in acetonitrile at room temperature in 80% yield.

The asymmetric addition of diethylzinc to aldehydes was performed in cyclohexane- hexane(1:1) at 0。C using 2.0 equiv of diethylzinc in the presence of 0.08 equiv of the chiral pyrrolidine (**2** or **3**) for 10-30 h to afford the secondary alcohols in 60-90% yields. The enantiomeric excess of the secondary alcohols was determined by optical rotation and 1H-NMR analysis of the corresponding Moscher's ester. The results were as follows; **2**: PhCHO, 95% ee(*R*); 4-chlorobenzaldehyde 90% ee(*R*); trans-cinnamaldehyde, 79% ee(**R**); hexanal, 55%ee(**R**), 3: PhCHO, 41%ee(**S**)) The *N*-methyl-4-hydroxypyrrolidine derivative (**2**) exhibited a high efficiency in chiral induction for the aromatic aldehyde up to 95% ee. However, hexanal could only be ethylated in moderate enantioselectivity (55% ee). On the other hand, enantiomeric excess

for the addition of diethylzinc to benzaldehyde using **3** was low (41% ee). Interestingly, a dramatic change of enantioselectivity was observed between **2** and **3**.

Publications:

1) Tomioka, K., Kanai, M. and Ikota, N.: Heterocycles, 42, 43-45 (1996).

2) Ikota, N. and Hama-Inaba, H.: Chem. Pharm. Bull., 44, 587-589 (1996)

3) Ikota, N., Hirano, J., Gamage, R., Nakagawa, H., and Hama-Inaba, H.: Heterocycles, in press.

3．1．BIO-MEDICAL SCIENCE Biochemistry and Biophysics

19　Induction of Antioxidative Activity in Rat Liver Microsomes Following Low Doses of Whole Body X-Irradiation

Osami Yukawa and Tetsuo Nakajima

Keywords: low dose radiation, radical scavenging activity, glutathione peroxidase, glutathione reductase, vitamin E, microsomal membranes

We have already reported that increases in total free radical scavenging capacity and glutathione reductase activity in rat liver cytosol are induced by low doses of whole body X-radiation, though no change is observed in the activities of superoxide dismutase and glutathione peroxidase and in the contents of glutathione and total SH group. We also showed that marked inactivation of the microsomal drug metabolizing enzyme system by higher doses of irradiation is significantly protected by the previous irradiation with low doses of X-rays. These results indicate that the protected decrease in the microsomal drug metabolizing enzyme activity might be due to the low dose radiation-induced increase in cytosolic antioxidative capacity to scavenge free radicals produced by irradiation. Drug metabolizing enzymes are specifically localized in microsomal membranes and easily inactivated by radical-induced membrane lipid peroxidation.

Therefore, in the present study, changes in rat liver microsomal antioxidative substances and enzymes were analyzed 1 day after whole body irradiation with low doses of X-rays to clarify further the relationship between intracellular antioxidative function and protection of membrane-bound enzymes. Irradiation of male Wistar rats with 1 to 20cGy causes a radiation dose-dependent increase in the radical scavenging activity in intact or detergent-solubilized microsomes, having a maximal activity at the dose of 5cGy. This changing pattern resembles closely the induction of cytosolic radical scavenging capacity by the same low doses of irradiation. Microsomal glutathione peroxidase activity determined using hydrogen peroxide or cumine hydroperoxide as a substrate showed a considerable increase after irradiation with 5cGy, suggesting that microsomal lipid peroxide formation is lowered by low dose radiation. Irradiation with 5cGy also brought about an increase in the content of vitamin E, a strong antioxidative substance bound to the membranes. In addition, microsomal lipid peroxide level determined as thiobarbituric acid reactive substances (TBARS) in rats irradiated with 1 to 20cGy was significantly lower than that in unirradiated rats, showing a mirror image of the pattern observed in the increase of microsomal radical scavenging activity by low dose radiation. This result suggests that microsomal lipid peroxidation is suppressed with increasing microsomal radical scavenging capacity. Furthermore, in vitro 50Gy-irradiation-induced lipid peroxidation was lower in liver microsomes prepared from 5cGy-irradiated rats than in those prepared from unirradiated rats, and liver cytosol prepared from 5cGy-irradiated rats was more effective to suppress radiation-induced microsomal lipid peroxidation, suggesting the presence of some co-

operative protection system against radiation between microsomal membranes and cytosol, such as the redox cycle of vitamin E and vitamin C. In conclusion, low dose in radiation induces cytosolic and membrane antioxidative activities to protect cellular functions against higher doses of irradiation.

2 0 Involvement of Diacyglycerol Production in Radiation in Radiation- induced Protein Kinase C Translocation in CUltured Rat Hepatocytes

Tetsuo Nakajima and Osamu Yukawa

Key words: rat hepatocyte, protein kinase C, lipid peroxidation, diacylglycerol, hydroxyl radical

We have demonstrated that radiation includes translocation of protein kinase C (PKC) from cytosol to membrane through membrane lipid peroxidation produced by radiation- induced active oxygens. Since PKC is a phospholipid-dependent protein kinase, it is possible that some lipid mediator might be related to the translocation. We investigated changes in lipid mediators after irradiation. Diacylglycerol(DAG) is a known endogenious PKC activator. Additionally, we analyzed increase of PKC molecules in hepatocyte membrane fraction using ［3H］PdBu binding assay in the previous experiment. As it is known that DAG binds to the phorbol ester binding site and PdBu is a kind of phorbol ester, we investigated effect of radiation on DAG production to elucidate whether or not DAG participates in radiation-induced PKC translocation. Mass content of DAG in rat hepatocytes was determined by converting DAG to ［32P］phosphatidic acid with［32P- γ］ATP and DAG kinase. The DAG content in irradiated rat hepatocytes increased 3min after irradiation in comparison with non-irradiated control.This result suggests that the rapid DAG production induces radiation-induced PKC translocation. Phospholipase C(PLC) is an enzyme that produces DAG. If DAG is produced by PLC after irradiation, a maximum increase in DAG content might be caused within a shorter period after irradiation. However it is difficult to measure changes in DAG within 3 min after irradiation due to a technical problem. Therefore, we used ・OH generating system (mixture of Cu(en)2, H2O2) to clarify radiation effect on DAG production. In fact, ・OH generating system induced DAG production in rat hepatocytes. The relationship between active oxygens and changes in phospholipids is under investigation to demonstrate the mechanism of radiation-induced PKC translocation.

Publication:

Nakajima, T. and Yukawa, O.: *Int. J. Radiol. Biol*. 70, 473-480, 1996.

21.Translational Pauses during the Synthesis of Proteins and mRNA Structure: Spider Silk Fibroin

Mitsuo Zama

Keywords: translation, mRNA structure, spider silk fibroin

It is known for several proteins that growing polypeptide chains being translated from mRNA transiently accumulate as discretely sized classes. This is due to pauses in the translational process. We have so far presented evidence to support the view that the mRNA secondary structure of the protein-coding region is responsible for the translational pauses observed for Bombyx mori silk fibroin, chicken type I collagen, colicin A, chloroplast photosystem II reaction center protein D1 and globin. Now, we have focused our attention on spider silk fibroin in this study.

Translational pauses are observed during spider fibroin syntesis. The spider major ampullate (dragline) silk of the spider Nephila clavipes is composed of multiple proteins. The amino acid sequences of the partial cDNA clones for the two major dragline silk fibroin components (Spidroin 1 and 2) exhibit repetitive motifs. Our detailed inspection of the nucleotide sequences of the repetitive motifs reveals highly selective site-specific codon usage patterns within a motif, suggesting that the secondary structure of the spider fibroin mRNA is optimized by the nucleotide sequence of the fibroin gene. The result, combined with our preceding results on silk fibroin from Bombyx mori suggest that translational pauses of spider silk may be interpreted in terms of the mRNA secondary structure.

Publication:

Zama, M.: Nucleic Acids Res. Symp. Ser., **37**, 179-180, 1997.

2 2．Radiation Effect on the Decay Rate of the Spin Probe in Whole Mice by Measuring *in vivo* EPR

Yuri Miura, Kazunori Anzai, and Toshihiko Ozawa

Keywords: in vivo EPR, radiation, nitroxyl radical, oxidative stress, reactive oxygen species

Radiation produces reactive oxygen species (ROS) in biological systems such as hydroxyl radical, superoxide and hydrogen peroxide, and causes various types of tissue damage due to successive free radical reactions. Organisms have protective systems against free radical reactions, e.g. endogenous antioxidants and antioxidative enzymes. Since these protective systems may well function in an association with each other, *in vivo* studies are important to accurately estimate radiation damage on organisms.

In vivo studies of radiation damage have been performed by invasive methods such as the measurement of lethal doses and tissue damages. These *in vivo* methods do not show the generation and reaction of free radicals, but rather the final results of free radical reactions. Therefore, a non-invasive *in vivo* method to measure free radical reactions is necessary to investigate the mechanisms of radiation damage in the whole body.

The *in vivo* EPR spectrometer enables the detection of both exogenous and endogenous free radicals in the whole body. The decay rates of a spin probe, nitroxyl radical, which is obtained from *in vivo* EPR measurements, were reported to be very susceptible to physiological redox state and oxidative stress, e.g., ischemia-reperfusion, g-radiation damage, aging, hypoxia and hyperoxia. Therefore, the decay rate of nitroxyl radical can be regarded as an indicator of the oxidative stress and the redox state in the whole body. In the present study, we examined the effect of X-irradiation on the decay rates of nitroxyl radicals in whole mice using *in vivo* EPR. One hour after irradiation, the decay rates of nitroxyl radical increased up to 15 Gy irradiation, but decreased over this dose.

The enhancement of the reduction rate of nitroxyl radical was suppressed by pre- administration of a radioprotector, cysteamine, suggesting that the enhancement of nitroxyl reduction was related to the radiation damage. Thiobarbituric acid-reactive substances (TBARS) in liver homogenate were increased by X-irradiation, indicating that X-irradiation induced oxidative stress in mice. Endogenous antioxidants such as a- tocopherol and ascorbic acid, and the activities of antioxidative enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase were not induced by X- irradiation under these experimental conditions, suggesting that the total reducing capacity of mice was not induced by X-irradiation. Therefore, it is speculated that oxidative stress induced by X-irradiation should be involved in the enhancement of the decay rates of nitroxyl radical.

Although the details of the mechanism which increased the decay rate are still unclear, oxidative stress caused by X-irradiation should be the major factor for this effect. The *in vivo* EPR system probing the nitroxyl reduction will continue to contribute to non-invasive *in vivo* studies of radiation damage.

Publications:

Miura, Y.,Anzai, K., Urano, S., and Ozawa, T.: Free Radic. Biol. Med. 23, 533-540, (1997).

Ozawa, T., Miura, Y., Anzai, K., and Urano, S.: *Food Factors for Cancer Prevention*, (Ohigashi, H., Osawa, T., Terao, J., Watanabe, S., Yoshikawa, T. eds.) Springer Verlag Tokyo, pp.448-451 (1997).

２３．Effects of Hydroxyl and Peroxyl Radicals on the Lipid Peroxidation and Ion Permeability of Membranes

Kazunori Anzai, Kunitaka Ogawa1, Yasuko Goto2, Jun-ichi Ueda, Yuri Miura, and Toshihiko Ozawa (1Kanagawa Univ., 2Kyoritsu College of Pharmacy)

Keywords: lipid peroxidation, liposome, ion selective electrode, hydroxyl radical, reactive oxygen species

Reactive oxygen species once generated in cells should affect the membranes and change the ion permeability of the membranes through reactions with membrane lipids and/or ion transporting proteins. In the present study, we have measured the effects of hydroxyl radical (·OH) generated by the reaction of Cu(en)2 with H2O2 and peroxyl radical (·OOR) generated from 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) on the lipid peroxidation and the membrane permeability of liposomes.
Liposomes (large unilamellar vesicles) were prepared by the reverse phase evaporation method using egg yolk phosphatidylethanolamine (PE)/egg yolk phosphatidylcholine (PC)
= 7:3 or 100% PC. Since PE was a product transesterified from PC, the acyl chain composition of PE was identical to that of PC. It was impossible to form liposomes with 100% PE. Lipid peroxidation was measured by observing the absorbance at 233 nm due to conjugated diene. K+ release from liposomes was measured with K+ selective electrode.
The generation of ·OH detected by the ESR spin trapping technique using 5,5-dimethyl- 1-pyrroline N-oxide as a spin trapping agent was dependent on the concentrations of Cu(en)2 and H2O2. When 100 mM H2O2 and 1 mM Cu(en)2 were used, the generation of ·OH was initially high but it decreased gradually and was almost zero at 1 h. When lower concentrations of H2O2 and Cu(en)2 were used, the ·OH generation proceeded constantly for more than 1 h . ·OH increased the amount of phospholipid hydroperoxide (LOOH) in PE/PC (7/3) liposomes. The LOOH increased with time and reached a plateau at 30 to 60 min. Larger amounts of ·OH, however, did not produce larger amount of LOOH. Rather, a larger amount of ·OH apparently made a smaller amount of LOOH. AAPH (20 mM), on the other hand, produced very little amount of LOOH in PE/PC (7/3) liposomes. On the contrary, when PC liposomes were used, AAPH produced more LOOH than ·OH did. K+ release from PC liposomes was significant when H2O2 (100 mM)/Cu(en)2 (1 mM) was applied, whereas no significant K+ release was observed when AAPH (20 mM) was used. Similar results were obtained for PE/PC (7/3) liposomes.
These results suggest that accumulation of LOOH is not responsible for the increase of the K+ permeability of the lipid membrane. The increase in the permeability of the lipid membrane may be due to other factors such as degradation of LOOH and modification of the head group of the phospholipids.

24. Gene Rearrangement of Interleukin-3 Gene by Insertion of Intracisternal A-particle Element in Radiation-induced Acute Myeloid Leukemia Cells in Mouse.

Hiroshi Ishihara, Izumi Tanaka

Keywords: retrotransposon intracisternal A-particle, myeloid leukemia, target duplication, interleukin-3 gene, genomic DNA cloning

Acute myeloid leukemia is induced in C3H/He inbred mice 1-2 years after whole-body irradiation of x-rays. An abnormal feature in the chromosomal structure and an increase in the genes for cytokines have been found in the leukemia cells. However, molecular evidence for occurrence of gene rearrangement has not been found. To find an abnormal structure in genes in the leukemia cells, genomes of cells from 7 leukemia strains of different origines corresponding to cytokine genes were molecularly compared with normal genome by genomic Southern analyses. We found gene rearrangement in one allele of interleukin-3 (IL-3) gene in the cells of leukemia strain L-8028(Fig.14(A)).

To investigate the rearrange allele of IL-3 gene, we constructed a genomic DNA library from the L-8028 leukemia cells and isolated both the normal and rearranged IL-3 gene. By comparison in the physical map and nucleotide sequence, it appeared that the abnormal allele was die to the insertion of intracisternal A-particle (IAP) element in size of 5.5kb into the promoter region of IL-3 gene (Fig. 14(b)). The IAP element is one the mouse retrotransposons and is structurally simila to the endogenous retrovirus that is constructed as the virus-like gag, pol and env genes interposed between two long- terminal repeat (LTR) sequences that contain promoter activity. Sequence analyses showed that the element is inserted into the locus by the retrotransposition mechanism that contains integrationof the complete IAP element following synthesis of the full-length IAP DNA element from short-sized IAP-RNA transcribed from IAP element.

From a conventional retrotransposition mechanism, the target genomic sequence in size of 4-8 bases was tandemly duplicated and located at both ends of the IAP element.

However, the length of target duplication was unusually long (82 base) in the IL-3-IAP structure. This suggested that the cohesive end with 82 base-single strand DNA was generated during integration.

Normal mouse possesses 1000 copies of the IAP element in the genome and the transcripts in cytoplasm. Although the retrotransposition has biological significance, it is hard to consider the contribution of the IAP in tumorigenesis from the current biological knowledge. Since the endogenous mutagenic structure was found in leukemia cells, further studies should be performed to analyze radiation-induced tumor in mouse.

Publication:

Tanaka, I. and Ishihara, H.: FEBBS Lett. 376, 146-150, 1995.

Figure Legend Fig. 14.

Southern blot analysis of genomes from myeloid leukemia cells. Normal germline DNA of C3H mice analyzed. EcoRI-digested DNAs were electrophorased, transferred to nylon membrane and hybridized with IL-3 DNA probe. Gene rearrangemant was found in cells from the L-8028 leukemia strain(*).

Physical map and sequence of the rearrangement of the rearranged IL-3 gene found in leikemia strain L-8028. IAP element was inserted into the promoter region of IL-3 gene with an usually long target duplication. Center lines represent mutated (Upper) and normal (lower) allele. Loci of the IAP sequence (double-underlined) was duplicated and placed at both ends of the IAP element (underlined).

25. Radiommunoassay of Steroid in the Serum with Excessive Amount of Lipids

Keiko Suzuki

Key words: radioimmunoassay, serum, steroid, lipid.

A very small amount of steroid in serum can be measured by radioimmunoassay. This experiment examined how to remove lipids which interfere with the assay.

Steroids are extracted from serum with organic solvents such as ethyl ether, but the lipids in the serum are also extracted by the solvent.Hitherto several methods have been employed to remove lipids from the sample bwefore the assay, for example, partitioning between 50% aqueous methanol and n-hexane, and column chromatography with Sephadex LH-20. However, the operations are complicated. A simple method was established as follows. The serum which had been stored at-80℃ was thawed and centrifuged at 16,000 rpm for 20 seconds. Most lipids were concentrated on the top, and the lower layer became clear. The radioimmunoassay of estradiol was performed with the lower clear phase of the serum without any trouble. It was supposed the lipids had been released from lipoprotein after freezing and thawing, although they had been solubilized in the original serim. The uniform distribution of estradiol between the lipid-rich layer on the top and the lower clear phase was confirmed as follows. 125I-Estradiol was added to the thawed serum and it was mixed for 15 hrs. at 4℃. The mixture was centrifuged, and 50 μl each of the top fraction and the lower solution were counted for radioactivity. It was found that there was no difference in the radioactivity of the two fractions. This means that the concentration of estradiol in the clear fraction represents the concentration in the whole serum.

In this method it is not necessary to correct for loss of quick, and is of wide application.

26. Comparison of LET Dependence for Cell Death and the Induction of Non-rejoining Chromatin Breaks in Normal Human Cells by Neon and Carbon Ion Beams

Masao Suzuki, Yoko Kase, Tatsuaki Kanai, Masami Watanabe1, Fumio Yatagai2 and Koichi Ando (1 Nagasaki University, 2 The Institute of Physical and Chemical Research)

Keywords: neon ion beam, carbon ion beam, PCC, non-rejoining chromatin break

We have investigated the LET dependence of cell death and the induction of non-rejoining chromatin breaks in normal human embryo cells irradiated by accelerated neon and carbon ion beams. Both ion beams were accelerated by the Riken Ring Cyclotron (RRC) at the Institute of Physical and Chemical Research in Japan. Chromatin-break induction was measured by counting the number of chromatin fragments detected by the premature chromosome condensation (PCC) technique. The yield of chromatin breaks per cell was estimated to be the number of PCC fragments in excess of the number of PCC fragments found in the non-irradiated cells. Non-rejoining chromatin breaks were measured as remaining fragments of PCC after 12-18 hours of post-irradiation incubation in a CO2 incubator at 370C.

The results indicated that cell death and the induction of non-rejoining chromatin breaks showed a qualitatively similar LET dependence. The LET-RBE curves for both cell death and the induction of non-rejoining chromatin breaks by neon ion beams had a broad peak in the LET range of 120 to 300 keV/ｵm and steeply downward trend up to 340keV/ｵm, while the curves by carbon ion beams showed a sharp peak of maximum efficiency of both effects in the LET range of 100 to 200 keV/ｵm. It seemed that the maximum effective position of the LET-RBE curves for both cell death and non-rejoining chromatin breaks by neon ion beams shifted to higher LET regions compared to the results by carbon ion beams (Fig.15). These results suggested that there was a good correlation between cell death and the induction of non-rejoining chromatin breaks in the case of both neon and carbon ion beam irradiations with different LETs.

Publication:

Suzuki, M., Kase, Y., Kanai, T., Yatagai, F. and Watanabe, M. : Int. J. Radiat. Biol., 72, 497-503, (1997).

3．2　BIO-MEDICAL SCIENCE Cell Biology

27．The Increase in the mRNA Level of Spermidine/Spermine N1 Acetyltransferase in HeLa Cells Accompanied with Growth Arrest

Sachiko Ichimura, Koei Hamana*, and Mitsuru Nenoi(* Gunma University)

Keywords: polyamine, putrescine, SSAT mRNA, ODC mRNA

Most living cells contain organic polycations, known as polyamines, which are essential for many growth related processes such as DNA replication, RNA synthesis and translation. In mammalian cells, putrescine, spermidine, and spermine are the main polyamines and the matabolic enzymes has been well characterized.

Interestingly, key enzymes of the pathway, ornithin-decalboxylase (ODC) and spermidine/spermine N1-acetyltransferase (SSAT) turn over the rapidly by the proteasomal degradation. SSAT, which catalyzes the acetylation of spermidine and spermine, might mediate some important functions in the repair process of cells damaged by radiation because it is known that the activity was increased by various inhibitors of cell proliferation. We report here that SSAT mRNA in an HeLa celll accumulated immediately after the growth arrest even in the normal process of culturing. Putrescine content in a cell increased remarkably in parallel with the SSAT mRNA accumulation.

HeLa S3 cells were seeded at low density (104 cells /cm) and the contents of DNA, RNA and polyamines were analized in the culturing process. Total mRNA levels of ODC and SSAT and the 18S rRNA level were estimated by radioactivities of the Northern hybridization and the RNA content per unit level of DNA was calculated as the relative value.SSAT mRNA level increased remarkably at the initial stage of growth arrest. The total content in each dish of polyamine, putrescine, spermidine or spermine, was determined by OPA method and the relative value per unit weight of DNA was calculated. Parallel to the increase of the steady state of SSAT mRNA, the acetylspermidine and putrescine contents in a cell increased remarkably at the beggning of the plateau phase. The induced SSAT would mediate the cellular conversion of spermine and spermidine to putrescine. The increase of putrescine as well as acetylspelmidine level may be an important celler process to introduce into the G0 stage.

Publication:

(1) Ichimura, S., Hamana, K. and Neoi, M.:Biochem. Biophys. Res. Comm. in press, 1998.

2 8.Higher Frequency of Concerted Evolutionary Events in Rodents than in Humans at the Polyubiquitin Gene VNTR Locus

Mitsuru Nenoi, Kazuei Mita, Sachiko Ichimura, and Akihiro Kawano

Keywords: polyubiquitin gene, concerted evolution, unequal crossover, numerical simulation

Ubiquitin is a highly conserved small protein of 76-aa functioning in the selective proteolysis of a variety of cellular proteins at the 26S proteasome, endocytosis of cell surface proteins,and NFKB signal transduction. It has been suggested that polyubiquitin genes, encoding tandemly repeated multiple ubiquitins, may show strong evidence of concerted evolution. A greater degree of homology between repeat units within a species as compared to orthologous repeat units in different species is a diagnostic feature of concerted evolution. However, in the case of polyubiquitin genes, data are not available for all loci in each species, and it is not clear whether the loci compared across species are orthologues or paralogues.

We have previously identified a high degree of homology in the 3'UTR between the polyubiquitin gene *UbC* of humans and the *CHUB2* of Chinese hamster, and showed that they share a pair of inverted repeats of 10 bp in length at the same location with the same sequence. In addition, we isolated another Chinese hamster polyubiquitin gene, *CHUB1*, which encodes five units, and showed both that its 3'UTR is highly homologous with that of the human polyubiquitin gene *UbB* and that a pair of inverted repeats is conserved at a different location and with a different nucleotide sequence from those of the *UbC* and the *CHUB2*. Based on these facts, it is quite reasonable to assume that both the relationship between the *UbC* and the *CHUB2* and between the *UbB* and the *CHUB1* are orthologous. This assumption was further supported by the observation that the sequence differences between *UbC* and *CHUB2* and those between *UbB* and *CHUB1* were evidently smaller than those between *UbC* and *CHUB1* as well as those between *UbB* and *CHUB2*.

We analyzed the concerted evolution of the polyubiquitin gene on the basis of the orthologous relationship of the *UbC* and *CHUB2* gene. That the mean of the synonymous sequence difference Ks, which is defined as the number of synonymous substitutions relative to the total number of synonymous sites, within the *UbC* and *CHUB2* genes (0.192±0.096) is significantly less than Ks between these genes (0.602±0.057) provided direct evidence for concerted evolution. Moreover, it also appeared that concerted evolutionary events have been much more frequent in the *CHUB2* gene than in the *UbC* gene, based on the observation that Ks within the *CHUB2* gene (0.022±0.018) is much less than that within the *UbC* gene (0.362±0.192), in spite of a higher rate of synonymous substitutions in rodents than in humans. In a numerical simulation, postulating that the major mechanism of concerted evolution in polyubiquitin genes is unequal crossing over, we showed that approximately 4000 unequal crossover events have occurred in the *CHUB2* gene since the divergence of humans and rodents 120 MYA (3.3x10-5 per year). In contrast, it appeared that unequal crossing over has occurred in the *UbC* gene no more than 60 times per 120 million years (5.0x10-7 per year).

Publication:

1) Neoi, M., Mita, K., Ichimura, S. and Kawano, A.: Genetics, 1998 in press

28. Hydrocortisone Is Involved in Regulating the Proliferation and Differentiation of Mouse Melanoblasts in Serum-Free Culture in the Presence of Keratinocytes

Tomohisa Hirobe

Keywords: melanocyte, keratinocyte, proliferation, differentiation, hydrocortisone

Mouse epidermal melanoblasts preferentially proliferated from disaggregated epidermal cell suspensions derived from newborn mouse skin in serum-free medium (MPM) supplemented with dibutyryl adenosine 3',5'-cyclic monophosphate (DBcAMP) and basic fibroblast growth factor (bFGF). After 12 days, almost all keratinocytes died, and pure and enriched culture of melanoblasts (ca.90%) and melanocytes (ca.10%) could be obtained. In order to clarify the role of hydrocortisone (HC) which is thought to be important for the regulation of melanocyte proliferation and differentiation, the hormone was added to MPM from the initiation of primary culture. The proliferation of melanoblasts was inhibited by HC in a dose-dependent manner and instead, most of the proliferating melanoblasts were induced to differentiate into melanocytes. A small number of pure melanoblasts derived from primary cultures at 12 days with MPM depleted of DBcAMP and bFGF were further cultured with MPM with or without HC in the presence of secondary keratinocytes that were subcultured from a pure population of primary keratinocytes in a serum-free medium. The inhibition of the proliferation of melanoblasts by HC as well as the stimulation of the differentiation of melanoblasts into melanocytes by HC was observed in the presence of keratinocytes, but not in their absence. Conditioned media or extracts prepared from pure keratinocytes in serum-free primary culture failed to replace the role of keratinocytes. These results suggest that HC plays an important role in the regulation of the proliferation and differentiation of mouse epidermal melanoblasts in culture in cooperation with factors supplied by keratinocytes.

Publication:

Hirobe, T.: Eur. J. Cell Biol., 71, 387-394, 1996.

29. Coodinate Change between Complement C1s Production and Chondrocyte Differentiation *in vitro*

Hisako Sakiyama, Koichi Makagawa, Kazuo Kuriiwa, Takeshi Fukuzawa, Misako Matsumoto1, Masaharu Takigawa2 and Toru Toyoguchi (1 The Center of Adult Diseases, 2 Okayama Univ.)

Keywords: complement C1s, chondrocytes, cell culture, differentiation, synthesis, active form specific antibody

The complement system plays an important role in the recognition of alien substances. C1s is an integral part of the first component of somplement C1, which initiates the classical pathway.The major synthesis site of serum C1s is the liver. However, C1s gene expression has been found in most other tissues, including brain, kidney, stomach, and in several types of cultured cells, such as monocytes, andothellial cells and fibroblasts. By immunostaining and *in situ* hybridization, we have shown increases in accordance with chondrocyte hypertrophy. We next examined *in vitro* synthesis of C1s usning hamster epiphyseal chondrocytes (HAC) and human chondrosarcoma cell line HCS-2/8. Hamster and human C1s produced by the cells were quantified by immunoblotting and sandwich enzyme-linked immunosarbent assay (ELISA), respectively. It was possible to measure active and inactive C1s by sandwich ELISA, when we used anti-human C1s monoclonal antibodies, M241 recognizing only active C1s, and M365 and M81 recongizing both active and inactive C1s. Approximately 40% of C1s secreted from HCS-2/8 was found to be activated in the culture medium, whereas C1s from HCS wasnot. C1s production increased in accordance with chondrocyte differentiation induced by ascorbic acid. In contrast, transforming growth factor beta 1 and basic fibroblast growth factor, which inhibited differentiation, suppressed C1s production. These results confirmed our previous observation showing that C1s sycthesis increased with differentiation into hypertrophic chondrocytes.

Publication:

1) Sakiyama, H., Nishida, M., Sakai, N., Nagino, K., Miyatake, S. and Imajo-Ohmi. S.:Intl. J. Cancer, 66, 768-771, 1996.
2) Toyoguchi, T., Yamaguchi, K., Nakagawa, K., Fukazawa, T., Moriya, H. and Sakiyama, H.: Cell Tissue Res. 285, 199-204, 1996.
3) Hidari, K.I.P.J., Ichikawa, S., Fujita, T., Sakiyama, H. and Hirabayashi, Y.J.Biol. Chem. 271, 14636-14641,1996.
4) Ichikawa, S., Sakiyama, H., Suzuki, G., Hidari, K.I.P.J. and hirabayashi, Y.:Proc. Natl.Acad. Sci.93, 4638-4643, 1996.
5) Wada E., Sakiyama, H., Nakamura, M. and Kanegasaki, S.: Cell Biochem. Func. 15, 19-26, 1997.
6) Sakiyama, H., Nakagawa, K., Kuriiwa, K., Imai, k., Okada, Y., Tsuchida, T. Moriya, H. and Imajoh-Ohmi, S.: Cell Tissue Res. 288, 557-565, 1997.
7) Nakagawa, K., Sakiyama, H., Fukazawa, T., Matsumoto, M., Takigawa, M., Toyoguchi,T. and Moriya, H.: Cell Tissue Res. 289, 299-305, 1997.

30. Production of Nitric Oxide by Rat Mammary Cells in Culture

Makoto Onoda and Hiroshi Inano

Keywords: nitric oxide, mammary cells, culture, LPS

Nitric oxide (NO) - a small, relatively unstable, diatomic free-radical - is a unique biological messenger molecule. This inorganic gas is synthesized from the amino acid L- arginine by the enzyme nitric oxide synthase (NOS), and is released as a metabolic product of diverse types of cells including endothelial, epithelial, neuronal and phagocytic cells. Its expanding range of functions includes controlling blood pressure by blood vessel relaxation, regulating blood clotting through the inhibition of platelet aggregation, acting as a neurotransmitter in the central and peripheral nervous system, and having a cytotoxic ability to kill tumor cells and intracellular parasites in the immune system. Apart from being an autocrine and paracrine mediator of homeostasis, NO has been found to inflict damage on important biomolecules and it was suggested to contribute to the cytocidal action of macrophages and to many pathological events. Recent reports in the literature also implicate overproduction of NO in inflammation, arthritis, myositis, and other diseases.

Meanwhile, in the last decade our research has dealt with the regulation mechanisms of the radiation-induced mammary tumorigenesis. Subsequently, it has prompted us to understand whether NO is associated with the initiation and/or promotion phase in the mammary tumorigenesis induced by radiation. In this context, we have undertaken isolation and culture of rat mammary cells to clarify NO-production ability of the mammary cells and to gain a firm clue to understand the relationship between radiation-induced mammary tumorigenesis and NO production in the mammary gland.

Mammary glands were aseptically isolated from the inguinal part of female Wistar-MS rats (8 to 9 weeks old) and digested by sequential enzymatic treatment to obtain the epithelial cell aggregates fraction. The aggregates (approximately 15 to 20 cells in each aggregate) were plated in the 24-multiwell plastic plate and cultured in 10% FCS/DMEM with 5% CO2-95% air at 37 ℃. Medium was replaced every 2 days with fresh 10% FCS/DMEM. On day 3 of culture, non-adherent cells were lysed by treatment with a hypotonic solution, and the culture was maintained with the same medium as described above. Constituent cell populations of the culture were assessed by immunocytochemical staining with specific antibodies against keratin (for epithelial cells), vimentin (for mesenchymal cells such as endothelial cells and fibroblasts) and α-actin (for smooth muscle cells). The presence of myoepithelial cells and epithelial cells also can be ascertained by performing alkaline phosphatase cytochemical staining with First Blue RR and neutral lipid droplet staining with Oil Red O, respectively. In a typical preparation of mammary cell cultures, the major cell population was epithelial cells of which purity was more than 70% of the confluent cell layer (Fig. 16). Remaining cell populations were mainly vimentin positive, and myoepithelial cells occupied less than 5% of total cell population. Because these results indicated that the mammary cell culture obtained essentially abounded in mammary epithelial cells, we decided to employ this culture system for further studies of NO-production by rat mammary cells in culture.

NO produced and secreted by mammary cells into culture medium was estimated by measuring NO2 converted from NO with Griess reagent after treatment with bacterial lipopolysaccharide (LPS, ~2 μg/ml)

and carboxy-PTIO (NO scavenger, 100 μM). NO secretion was detectable after 6 h lag period and increased remarkably in a time- dependent manner to 48 h of culture period (Fig.17). The secretion of NO from mammary cells cultured with LPS increased in a dose-dependent manner up to a concentration of 0.5 μg/ml, and was slightly inhibited at higher concentrations of LPS up to 2 μg/ml. The reason for decline of NO secretion may be due to cytotoxicities of LPS and/or NO overproduced from mammary cells with the LPS-stimulation. These findings suggest that the culture contains at least a certain cell population that responds to LPS- stimulation and then it produces NO.

NO secretion from mammary cells with LPS-stimulation was inhibited by addition of glucocorticoids such as hydrocortisone and corticosterone, inhibitors of inducible NOS isozyme (iNOS), in a dose-response manner. Secretion of NO was mostly inhibited with glucocorticoids at 1 μM, and median inhibition doses (ID50) of hydrocortisone and corticosterone were 40 nM and 150 nM, respectively. These results indicate that NO production by mammary cells with LPS-stimulation is mediated through iNOS induction or activation.

Furthermore, we attempted to localize NO-producing cells by performing iNOS immunocytochemistry with a specific antibody and cytochemistry of NADPH-diaphorase that is identical to NOS. Positive staining of iNOS was detected in only a few cells that exhibited spindle-like shape and were scattered in the culture. Since the epithelial cell population was the major cell population and occupied more than 70% of total cells in the culture, it is unlikely that mammary epithelial cells express iNOS after LPS- stimulation. Therefore, other types of cells in the mammary gland may contribute to production of NO in an attack of bacteria and acute inflammatory response.

Although production and secretion of NO in the mammary gland after bacterial infection are now certain, NO-producing cell populations that express iNOS in the glands still remain to be clarified. In addition to iNOS, other types of NOS isozymes, constitutive NOS, may contribute to physiological functions of mammary gland. Moreover, continued exposure to NO over long periods of time may lead to accumulation of populations of cells with activated oncogenes or impaired tumor suppresser genes. Therefore, our next approach in this project is to elucidate NO-producing cell populations in the mammary gland after exposure to radiation.

Fig. 16. Photomicrograph of rat mammary cells isolated from female Wistar-MS rat mammary glands by sequential enzymatic digestion. Bar=100 μm.

Fig. 17. Nitric oxide production by cultured rat mammary cells. The cultures were treated with LPS (0.5 m μg/ml) and carboxy-PTIO (100 μM), and incubated for 48 h. Conditioned medium samples were collected at indicated times and NO2 determination was carried out by Griess reagent.

31. Production of Nitric Oxide by Rat Mammary Cells in Culture

Makoto Onoda and Hiroshi Inano

Keywords: nitric oxide, mammary cells, culture, LPS

Nitric oxide (NO) - a small, relatively unstable, diatomic free-radical - is a unique biological messenger molecule. This inorganic gas is synthesized from the amino acid L-arginine by the enzyme nitric oxide synthase (NOS), and is released as a metabolic product of diverse types of cells including endothelial, epithelial, neuronal and phagocytic cells. Its expanding range of functions includes controlling blood pressure by blood vessel relaxation, regulating blood clotting through the inhibition of platelet aggregation, acting as a neurotransmitter in the central and peripheral nervous system, and having a cytotoxic ability to kill tumor cells and intracellular parasites in the immune system. Apart from being an autocrine and paracrine mediator of homeostasis, NO has been found to inflict damage on important biomolecules and it was suggested to contribute to the cytocidal action of macrophages and to many pathological events. Recent reports in the literature also implicate overproduction of NO in inflammation, arthritis, myositis, and other diseases.

Meanwhile, in the last decade our research has dealt with the regulation mechanisms of the radiation-induced mammary tumorigenesis. Subsequently, it has prompted us to understand whether NO is associated with the initiation and/or promotion phase in the mammary tumorigenesis induced by radiation. In this context, we have undertaken isolation and culture of rat mammary cells to clarify NO-production ability of the mammary cells and to gain a firm clue to understand the relationship between radiation-induced mammary tumorigenesis and NO production in the mammary gland.

Mammary glands were aseptically isolated from the inguinal part of female Wistar-MS rats (8 to 9 weeks old) and digested by sequential enzymatic treatment to obtain the epithelial cell aggregates fraction. The aggregates (approximately 15 to 20 cells in each aggregate) were plated in the 24-multiwell plastic plate and cultured in 10% FCS/DMEM with 5% CO2-95% air at 37 ℃. Medium was replaced every 2 days with fresh 10% FCS/DMEM. On day 3 of culture, non-adherent cells were lysed by treatment with a hypotonic solution, and the culture was maintained with the same medium as described above. Constituent cell populations of the culture were assessed by immunocytochemical staining with specific antibodies against keratin (for epithelial cells), vimentin (for mesenchymal cells such as endothelial cells and fibroblasts) and α-actin (for smooth muscle cells). The presence of myoepithelial cells and epithelial cells also can be ascertained by performing alkaline phosphatase cytochemical staining with First Blue RR and neutral lipid droplet staining with Oil Red O, respectively. In a typical preparation of mammary cell cultures, the major cell population was epithelial cells of which purity was more than 70% of the confluent cell layer (Fig. 16). Remaining cell populations were mainly vimentin positive, and myoepithelial cells occupied less than 5% of total cell population. Because these results indicated that the mammary cell culture obtained essentially abounded in mammary epithelial cells, we decided to employ this culture system for further studies of NO-production by rat mammary cells in culture.

NO produced and secreted by mammary cells into culture medium was estimated by measuring NO2 converted from NO with Griess reagent after treatment with bacterial lipopolysaccharide (LPS, ~2 μg/ml)

and carboxy-PTIO (NO scavenger, 100 μM). NO secretion was detectable after 6 h lag period and increased remarkably in a time-dependent manner to 48 h of culture period (Fig.17). The secretion of NO from mammary cells cultured with LPS increased in a dose-dependent manner up to a concentration of 0.5 μg/ml, and was slightly inhibited at higher concentrations of LPS up to 2 μg/ml. The reason for decline of NO secretion may be due to cytotoxicities of LPS and/or NO overproduced from mammary cells with the LPS-stimulation. These findings suggest that the culture contains at least a certain cell population that responds to LPS-stimulation and then it produces NO.

NO secretion from mammary cells with LPS-stimulation was inhibited by addition of glucocorticoids such as hydrocortisone and corticosterone, inhibitors of inducible NOS isozyme (iNOS), in a dose-response manner. Secretion of NO was mostly inhibited with glucocorticoids at 1 μM, and median inhibition doses (ID50) of hydrocortisone and corticosterone were 40 nM and 150 nM, respectively. These results indicate that NO production by mammary cells with LPS-stimulation is mediated through iNOS induction or activation.

Furthermore, we attempted to localize NO-producing cells by performing iNOS immunocytochemistry with a specific antibody and cytochemistry of NADPH-diaphorase that is identical to NOS. Positive staining of iNOS was detected in only a few cells that exhibited spindle-like shape and were scattered in the culture. Since the epithelial cell population was the major cell population and occupied more than 70% of total cells in the culture, it is unlikely that mammary epithelial cells express iNOS after LPS-stimulation. Therefore, other types of cells in the mammary gland may contribute to production of NO in an attack of bacteria and acute inflammatory response.

Although production and secretion of NO in the mammary gland after bacterial infection are now certain, NO-producing cell populations that express iNOS in the glands still remain to be clarified. In addition to iNOS, other types of NOS isozymes, constitutive NOS, may contribute to physiological functions of mammary gland. Moreover, continued exposure to NO over long periods of time may lead to accumulation of populations of cells with activated oncogenes or impaired tumor suppresser genes. Therefore, our next approach in this project is to elucidate NO-producing cell populations in the mammary gland after exposure to radiation.

Fig. 16. Photomicrograph of rat mammary cells isolated from female Wistar-MS rat mammary glands by sequential enzymatic digestion. Bar=100 μm.

Fig. 17. Nitric oxide production by cultured rat mammary cells. The cultures were treated with LPS (0.5 m μg/ml) and carboxy-PTIO (100 μM), and incubated for 48 h. Conditioned medium samples were collected at indicated times and NO2 determination was carried out by Griess reagent.

3 2．Effects of Reoxygenation on Repair of Potentially Lethal Radiation Damage in Cultured MG-63 Osteosarcoma Cells

Koichi Ando, Masayuki Iizuka, Takashi Aruga, Yoshiya Furusawa, Hiromi Itsukaichi, Kumiko Fukutsu, Hideko Nagasawa and Hideshige Moriya

Keywords: hypoxic,delayed plating, linear qudratic, colony formation

Effects of reoxygenation on repair of potentially lethal radiation damage were investigated using MG-63 human osteosarcoma cells in vitro. When exponentially growing MG-63 cells were hypoxically cultured for 24 hours, cells stopped growing and remained at a sparsely-plated density. The hypoxic cells were then reoxygenated by exposure to air, and irradiated with a single dose of 3 Gy of X-ray irradiation. The fraction of the reoxygenated cells surviving 3 Gy increased by a factor of 20.6 when the colony assay was delayed by 24 hours. In control cells which were cultured under aerobic conditions only before receiving a single dose of 3 Gy, the delayed plating assay increased the surviving fraction by a factor of 2.5. The difference in magnitude of the repair observed between reoxygenated and aerobic cells was less prominent in densely-plated, confluent cells. The enhanced repair after reoxygenation was mainly due to a decrease of the αvalue of the fit of the dose-survival curve to the linear-quadratic model, whereas the most significant change in the fit of the dose-survival curve for the aerobic cells was a decrease in the βvalue. The control aerobic cells accumulated at the G2/M phase after irradiation whereas the reoxygenated cells did not show such accumulation. When the hypoxic cells were irradiated and then reoxygenated, repair of these hypoxically-irradiated cells was also enhanced. This is the first report to imply that reoxygenation could increase cell survival after tumor irradiation.

Publication: Iizuka, M., Ando, K., Aruga, T.,Itsukaichi, H., Fukutsu, K., Nagasawa, H. and Moriya, H.:Radiation Research 147, 179-184, 1997

33. Optimal schedule of heavy iron radiotheraspy on the biological basis - Biological evaluation of clinically used-ridge filter for human lung cancer cell lines -

Tadaaki Miyamoto, Yasuo Takiguchi, and Hiroshi Matzuzaki

Key word: carbon beam, lung cancer cell line, RBE, SOBP, ridge filter

The purpose of this reseach project is to investigate experimentally the optimal dose and fractionation in heavy iron therapy . As the first step of the project, we aim to demonstrate RBE of human tumor cells for carbon beam at various dose-average LET by using clinically used-ridge filter . In 1995, we reported RBE of human lung cancer cell lines for 290MeV carbon beam at 80KeV/μm in comparison with that for 30MeV/μm fast neutron. In the present study(1996), we evaluated the biological response of these cell lines within 6cm-spread out Bragg peak (SOBP)of 290MeV carbon beam .(Materials and Methods) For experiment, four human cell culture lines(2 adenocarcinoma,1 squamous cell carcinoma,1 large cell carcinoma) were used. The cells were exposed to 290MeV carbon beam at 13,40,50 and 80 KeV/μm and to 200 keV X-ray ,and their dose-survival curves were obtained by colony forming method.(Results) 1) As LET of carbon beam increased,RBE increased for all cell lines. 2) As shown in Fig.1, the relative biological dose(physical dose x RBE at 10% survival level) tended to decrease for all cell lines as LET increased from 40 to 80 KeV/μm. 3) The physical doses of X-ray at 10% survival level were well-correlated with that of carbon beam at 80 KeV/μm by a factor of 2,although each cell line showed different sensitivity to the both beams. It means that RBE of all human lung cancer cell lines for carbon beam was 2. (Discussion) The uniformity of biological dose of carbon beam in SOBP designed by clinically used ridge filter was evaluated by using four human lung cancer cell lines. As a result, it decreased toward the depth of SOBP by about 5% as LET of carbon beam increased. It suggests that the desigh of righe filter now clinically used should be modified at least for lung cancer .

3 4．The limited operation combined with heavy ion particle radiotherapy for breast cancer

Hiroshi Matzuzaki, K. Isono, Y. Miyazawa and T. Miyamoto

Key word: BCT, breast cancer cell line, carbon beam, RBE

Nowaday, breast conservative therapy (BCT) comes to prevail in our country, in which a radiotherapy to breast is a key method to prevent local recurrence .However, intraductal carcinoma and/or intraductal component in invasive carcinoma are associated with a high incidence of breast recurrence because of the radioresistance and restrict the usefullness of BCT. Heavy ion particles have strong biological activity as well as excellent dose localization,so that a radioresistant tumor would be more effectively controlled by them. The purpose of present study is to clarify the biological activity of carbon beam against human breast carcinoma in comparison with X ray . For experiment, 3 culture cell lines and one nude tumor derived from human breast cancer were used. Cells survival in vitro after irradiation was determined by using colony forming method and that in vivo by regrowth assay method. Dose response curves were obtained against X ray and carbon beam (290MeV at 80KeV/μm for culture cells and at 50KeV/μm for nude line),respectively . Relative biological effectiveness(RBE) at 10% survival level was obtained from these survival curves(Tabel 1). RBE at 80KeV/μm for 3 cell lines was 2.59
±0.30 in average whearas RBE at 50KeV/μm for a nude tumor was 1.72 in single treatment and 2.23 in three fractionated treatment. These RBE values of breast cancer lines were higher than those of lung cancer lines(RBE:2), suggesting the promising usefulness of carbon beam in the treatment of breast cancer .

Table 1. RBE of breast cancer cell line for 290Mev Carbon Beam

	cell line	RBE (10% survival)
in vitro (80KeV)	OCUB-M CRL1500 YMB-1	2.89 (4.74 / 1.64) 2.73 (3.66 / 1.34) 2.17 (2.91 / 1.34)
in vivo (50KeV)	CMM-1	1.72 (12 / 6.96)

3 5．Studies of Radiosensitivity in Human Hepatoma Cell Lines for Heavy Charged Particle Therapy

Tadamichi Denda1, Masaharu Yoshikawa2, Hirotoshi Kato, Junichi Fujita, YOshiya Furusawa, Tadaaki Miyamoto, Hirohiko Tsuji, and Hiromitsu Saishio2 (1 Chiba Cancer Center Hospital ; 2 Chiba Univ., School of Medicine)

Key words: rediosensitivity, heavy charged particle therapy, hepatocellular carcinoma, HLE, HLF, nuclear damage assay (NDA), MTT assay

In this study, we studied radiosensitivity of the hepatocellular carcinoma to heavy charged particle therapy using human hepatoma cell lines, HLE and HLF. Several votles of cultured cells were cultivated for 24,96,192,288 and 384 hours, respectively, after being irradiated by 12C ion beam (75KeV/μm). Then, they were subjected to nuclear damage assay (NDA) and MTT assay.Nuclear changes caused by anticancer drugs can be detected by NDA. MTT assay is used to observe the changes of the activity of succinate dehydrogenase. In addition, we examined ladders of DNA for the purpose of detecting apoptosis.
Since 1991, we have studied a chamosensitivity test by which it is possible to predict the efficacy of anticancer drugs. Examination items are NDA and MTT assay. For liver cancer and pancreatic we chose some anticancer drugs, mainly based on the results od NDA. MTT assay is mainly applied to gastric cancer. The same technique as for the chamosensitivity test was applied in this study to the radiosensitivity test. The following are the reaults.
Various changes in NDA were observed in HLE cultivated for 4hours, including karyopyknosis, karyorrhexis, lobulation and ununiformity of nuclear staining.
As exposure doses were increased, enzyme activities decreased above 96 hours cultivation in MTT assaay.
DNA ladders were not detected 24 hours after irradiation at any exposure dose.
Significant differences were recognized in HLE cells cultivated for a short term, 4 hours, in NDA with which the change of cell nuclei could be observed.

3．3．BIO-MEDICAL SCIENCE Immunology and Hematology

36．Cytogenetic Analysis of Thymocytes during Early Stages after Irradiation in Mice with Different Susceptibilities to Radiation-Induced Lymphomagenesis

Ying Chen*, Eiko Kudo, Toshihiko Sado, Masahiko Muto (* China Institute for Radiation Protection)

Keywords: chromosome aberration, strain difference, genomic instability, radiaition- induced llymphomagenesis

It is known that exposure of B10.Thy1.1 mice to fractionated X-irradiation induces a high incidence (> 95%) of thymic lymphomas, whereas the incidence of STS/A mice was very low (<8-9%). Such strain differences presumably are determined genetically, and various genetic factors have been reported to be involved in radiation-induced lymphomagenesis.

The mechanism of radiation-induced lymphomagenesis appears to develop through a complex and multistep process. Specific alterations in particular chromosomes are associated, with different degrees of consistency, with specific types of tumors, or with neoplasma in general. Trisomy 15 is frequently found in mouse T cell lymphomas arising spontaneously or following induction by irradiaiton or chemical carcinogens. We have also indicated that the aberrations / translocations such as trisomy 15,t(7F;10C), t(1A;13D) or t(6A;XB), which occured in single progenitor cells at early T cell differentiation just before or after γT cell receptor (TCR) rearrangements, might be important candidates for initiating events in about half of the individually irradiated mice. In the other half of the individually irradiated mice, microgengetic changes were suggested to be initial events and also might take place in single progenitor cells just before or after γTCR rearrangements.

To investigate the relationship between radiaiton-induced chromosomal instability and lymphomagenesis, the incidences of chromosome sberrations and of cells with abnormal chromosome complements in the thymuses of B10.Thy1.1 and STS/A strains which have shown marked diferences in susceptibility to radiation-induced lymphomagenesis were compared at an early time (12-33 days) after leukemogenis whole-body X-irradiation.

Numerical and structure aberrations of chromosome in B10.Thy1.1 mice were higher than those in STS/A mice. The average incidence of trisomy 15 was 37.5% (12/32 mice) from 12 days to 32 days after irradiation in B10.Thy 1.1 mice, whereas none was found (0//8 mice)in STS/A mice. Frequencies of aberrations in chromosomes 15, 12, 11 of the thymocytes from irradiated B10.Thy1.1 mice were higher than those from irradiated STS/A mice, and especially, the most frequent translocations occured in the E-F regions of chromosome 12.

Using B10.Thy1 congenic mice, we found that in many cases (8/13), the chromosome aberrations

detected in the thymocytes from B10.Thy1.1 mice at an early time after irradiation could also be observed in the donor-type (Thy1.1) lymphomas developed from recipient mice 4-5 months after intrathymic injection, suggesting that these chromosome aberrations have some role in lymphomagenesis. The difference of chromosome instability detected at an early time after irradiation might be due to genetic background with regard to genomic instability induced by radiation.

Publication:

1) Muto, M., Chen, Y., Kudo, E., and Mita, K.: Jpn. J. Cancer Res.,87, 247-257, (1996).

2) Chen, Y., Kudo, E., Sado, T. and Muto, M.: J. Radiat. Res., 37, 267-276 (1996).

37. Cellular Basis for Strain-Dependent Thymic Lymphomagenesis after Fractionated Whole-Body X-Irradiation

Hitoko Kamisaku and Shiro Aizawa

Key words: fractionated radiation, bone marrow chimeras, thymic lymphoma

We previously reported that B10 strain mice are extremely susceptible to induction of thymic lymphomas by fractionated whole-body X-irradiation (FX), whereas C3H mice are fairly resistant. Furthermore, our data indicate that a depletion of pre T cells in the bone marrow combined with atrophy of the thymus in the irradiated mice is necessary, but not sufficient for development of thymic lymphoma, since irradiation severely depleted the pre T cells in the bone marrow and the spleens of both lymphoma induction susceptible and -resistant strain mice.

In order to explore the cellular basis for strain-dependent thymic lymphomagenesis after FX-treatment, we used radiation bone marrow chimeras in which thymic lymphoma- induction-susceptible B10.BR (H-2K) and -resistant C3H (H-2K) mice were lethally irradiated and reconstituted with bone marrow of C3H or B10.BR donor mice, respectively, or with a mixture of bone marrows of both strain mice. B10.BR→C3H bone marrow chimeras had a high incidence of thymic lymphomas after FX-treatment as did B10.BR → B10.BR chimeras, whereas C3H → B10.BR shimeras had a low incidence as did C3H → C3H chimeras. Furthermore, FX-treatment of [B10.BR +C3H] → B10.BR mixed chimeras induced similar numbers of thymic lymphomas which were originated from B10.BR or C3H bone marrow cells, respectively.

These results indicate that the susceptibility of bone marrow chimeras to FX-induced thymic lymphomagenesis is determined by the origin of bone marrow cells and they suggest that bone marrow-derived cells other than pre T cells have a cricial role in the development and/or supression of lymphomas induced by irradiation.

Publication:

Kamisaku, M., Aizawa, S., Kitagawa, M., Ikarashi Y., and Sado, T.: Int. J. Radiat. Biol. 72, 191-199, 1997.

38. The Mechanism for Inhibition of Radiation-Induced Myeloid Leukemia by Caloric Restriction

Kazuko Yoshida, Yoko Hirabayashi* and Tohru Inoue* (*Cellular and Molecular Toxicology, NIHS)

Keywords: radiation-induced myeloid leukemia, caloric restriction, hematopoietic stem cells, cell cycle

The spontaneous incidence of myeloid leukemia in C3H/He male mice is 1%, and the incidence increases to 23.3% when a 3 Gy whole-body X-ray irradiation was given.However, the incidence of myeloid leukemia was found to be significantly decreased by caloric restriction; it was reduced to 7.9% and 10.7% when restriction was started before, (6 weeks old) and after (10 weeks old) irradiation, respectively. In addition, the onset of the myeloid leukemia in both restricted groups was prolonged to a greater extent as compared with the control diet group. Numbers of hematopoietic stem cells, the possible target cells for radiation-induced leukemia, in the groups for the calorie restriction demonstrated a significant decrease, especially in the spleen, as compared with that in the control, when the evaluation was made at the time of radiation exposure. However, the incidence of myeloid leukemia also decreased when the caloric restriction was started after radiation. In this case, the number of target cells was the same as in the control group, therefore, the findings were not due to only the number of target cells. Then, we examined the cell cycle of these stem cells.We evaluated the kinetics of CFU-S by means of newly developed BUUV method which allows us to evaluate cycling fraction at steady-state hemopoeisis of the both control and restriction groups. Bromodeoxyuridine (BrdUrd) incorporated into cells in S-phase shows no cytotoxicity; however the cells that have incorporated the label are killed with one exposure to near-ultraviolet (UV) light. Thus the total number of stem cells that are cycling, other than dormant CFU-Ss, can be measured by continuous labeling with BrdUrd through an osmotic pump.The control and restricted diet mice were implanted subcutaneously with an osmotic pump (ALZA Corp., Palo, CA) which allows continuous infusion of BrdUrd at a dose rate of 1mg /Kg/h. The osmotic pumps were kept in both mice for 5 days. Then, bone marrow and spleen cells from control and restricted mice were harvested and exposed to a near- UV illuminator (Model UVP, Funakoshi Co., Japan) with UV wave lengths longer than 300nm, and a peak at 365 nm. The UV-dose of exposure was 4000J/m2. Then the cells were injected into lethal irradiated mice for CFU-S assay. In the femur, the size of cycling fraction of CFU-s in the restricted groups (26%±4.5) was fewer than on the control group (44%±20.) In the spleen, it was also fewer in the restricted group(18.7%±2.9) than control group (25.6%±3.5) Those results imply that hematopoietic stem cells from restricted mice proliferate at a rate nearly as slow as those from control diet mice.
Therefore, the cell cycle kinetics of hematopoietic stem cells may also participate in the inhibition of radiation-induced myeloid leukemia.

Publications:

1) Yoshida, K., Inoue, T., Nojima, K., Hirabayashi, Y. and Sado, T. : Proc. Natal. Acad. Sci. USA 94, 2615-2619, 1997
2) Yoshida,K., Inoue,T., Hirabayashi, Y., Matsumura,T., Nemoto,K. and Sado,T. : Leukemia, 11, Suppl. 3, 410-412,1997

39. Loss of CXCR4 Expression During Positive Selectioj in the Thymus

Gen Suzuki, Yuriko Nakata, Akiko Uzawa, Toshiyuki Saito, Kazuei Mita and Takuji Shirasawa*

(*Tokyo Metropolitan Institute of Gerontology)

Key words: CXCR4 expression, SDF-1, Tlymphocyte, development, mouse

SDF-1 is a member of CXC chemokine. In contrast to other chemokines that are induced by inflammation, SDF-1 is constitutively produced by stromal cells. In order to investigate the physiological roles of SDF-1, we constructed a fusion protein between murine SDF-1α and the constant region of human IgG(SDF-1-Cγ1)for detecting SDF-1 receptor, CXCR4, by an immunofluorescence technique. SDF-1-γ1 stained EL-4 Tlymphoma cells and the staining was blocked by recombinant human (rh) SDF-1β.The expression levels of CXCR4 altered along with the T cell maturation. Most c-kit+ hematopoietic precursors in fetal liver in gestational day(DG) 14.5 embryo were CXCR4-, while c-kit+ double negative (DN) thymocytes in the embryo were CXCR4lo. CXCR4 expression increased along with T cell maturation up tp double positive (DP) cell stage; CD3- DP cells in GD16.5 embryo and adult mice were CXCR4+. Interestingly, CXCR4 expression was down-modulated after positive selection(Fig. 19); CD3hi-DP and -single positive (SP) thymocytes were CXCR4. Circulating T cells in peripheral blood were CXCR4-/lo, but T cells regained CXCR4 expression when they landed on peripheral lymphoid tissues. After activation with ConA, T cells down-modulated CXCR4 again.

These results suggest that CXCR4 works as an anchor for T cells to stay in SDF-1- expressing tissure by controlling the CXCR4 expression during development and activation.

Publication:

Nomura, M., Matsuda, Y., Itoh, H., Hori, T., and Suzuki, G.:Cytogenet. Cell Genet.73, 286- 289, 1996

Fig. 19. CXCR4 expression is down-regulated upon positive selection. A: Adult thymocytes consist of CXCR4-/lo and CXCR4+ cells. B,C: CD4 and CD8 profiles are depicted for whole thymocytes (B) or CXCR4- thomocytes that are sorted by FACStar (C). Purify of sorted cells was about 85%. Note that SP cells are enriched in CXCR4- population. D: Ag8.653 supernatant was used as a control. E: Double staining of whole thymocytes with SDF-1-Cγ1(FITEC) and anti-CD3ε(PE).

40. IL-1 Skews the Differentiation of Helper T Cells by Interfering with Peripheral Tolerance

Yukiko Nakata, Akiko Uzawa and Gen Suzuki

Key words: co-stimulation, IL-1, CTLA-4, Th1/Th2 balance

Antigen dose is one factor that controls the balance between type 1(Th1) and type 2 (Th2) helper T(Th) cells. Low doses of Staphylococcal Enterotoxin B (SEB) induced IFN- γ- and IL-4-producing type 0 (Th0)-like cells, while intermediate doses of SEB induced Th1 cells in vivo. High doses of SEB induced activation induced cell death (AICD) and anergy of SEB-reactive Vβ8+ CD4 T cells, which hampered the investigation of Th phenotypes. In order to interfere with AICD and anergy, we administrated IL-1 after SEB inoculation. Vβ8+ CD4 T cells from mice receiving high doses of SEB plus IL-1 differentiated to Th0-like cells not only in wild type B6 mice but also in β2-microglobulin deficient mice. Thus, IL-4 from NK1.1+ T cells was dispensable for the differentiation of Th0-like cells. Although CTLA-4 is a negative regulator of IL-2 production, Il-1 and cross- linking of CTLA-4 co-stimulated Vβ8+ CD4 T cells both for IL-4 production and proliferation. Moreover, IL-1 enhanced IL-6 production in vivo, which might favor IL-4 production from conventional Th cells. These results indicate that IL-1 modulates Th1/Th2(Th0) balance through interfering with peripheral tolerance and augmenting IL-4 production from CTLA-4 expressing Th0/Th2 cells (Fig.20).

Publications:

1) Nakata, Y., Matsuda, K., Uzawa, A., Nomura, M., Akashi, M.and Suzuki, G.:J. Immunol. 155, 4231-4235, 1995.
2) Suzuki, G., Nomura, M., Uzawa, A., Akashi, M., and Nakata, Y.: Int. Immunol.7, 37-43, 1995.
3) Taki, S., Sato, T., Ogasawara, K., Fukuda, T., Sato, M., Hida, S., Suzuki, G., Mitsuyama, M., Furuta, T, T., Hojima, S., Taniguchi, T., and Asano, Y.: Immunity 6, 673-679, 1997.

Fig. 20. Synergistic effort of IL-1 and CTLA-4 co-stimulation on the production of IL-4, IL-10 and IFN-γ. Preactivated T cells were stimulated with the combination of plate-coated antibodies against TCR Vβ8, CD28, and CTLA-4 in the presence (gray column) or absence (black column) of rhIL-1(100 unit/ml). Note that IL-1 partially overcomes the negative effect of CTLA-4 in the production of IFN-γand IL-10.

41. Flow Cytometric Analysis of Hematopoietic Cells in Irradiated Mice Treated with a Heat-killed *Lactobacillus casei* Preparation

Masako Furuse and Kazuko Tsuneoka

Keywords: Lactobacillus casei, flow cytometry, granulocyte, lymphocyte, radioprotection

We reported previously that a single subcutaneous injection of a heat-killed Lactobacillus casei preparation (LC 9018), given after irradiation, increased the survival rate of lethally irradiated mice. LC 9018 also stimulated the recovery of leukocyte numbers in peripheral blood and hematopoietic progenitor cells in hematopoietic tissues.

In this study, to elucidate which lineage-specific cell population contributed most to the protection of lethally irradiated mice from bone marrow death, we analyzed in detail the hematopoietic cells using flow cytometry. On the 13th day after irradiation, which is a critical time for bone marrow death, the percentage of granulocytic cells (Gr-1 and Mac-1 positive cells) was high in the LC-9018 treated mice. In the peripheral blood, about 50% of the leukocytes were taken up by granulocytic cells. In both the spleen and bone marrow, the percentage of granulocytic cells was high. On the other hand, in the saline treated mice, the percentage of granulocytic cells in the peripheral blood, the spleen and especially the bone marrow was low.

Next, we analyzed the change in the cell distribution in the peripheral blood of irradiated mice from the 1st day to the 21st day after irradiation. In the LC-9018 treated mice, the recovery of the granulocytic cell population was remarkably accelerated (Fig.21). It increased to four times the normal level on the 13th day after irradiation. In contrast, in the saline treated mice, in spite of an increase in granulocytic cell number observed on the 8th day, a decrease was observed on the 13th day. The T-lymphocytic cell population in the LC-9018 treated mice recovered slowly in a manner similar to that observed in the saline treated mice. The B-lymphocytic cell population also recovered slowly. However,the B-lymphocyte cell population in the saline treated mice was five times that in the LC- 9018 treated mice.

To establish whether the T-lymphocyte population contributed to the radioprotection of irradiated mice from bone marrow death, we injected LC 9018 into irradiated athymic nude mice. The administration of LC 9018 significantly increased the 30-day survival rate of lethally irradiated athymic nude mice (88%) compared with that of saline (25%). Flow cytometric analysis of hematopoietic cells on the 13th day after irradiation showed a stimulation of granulopoiesis in the LC-9018 treated athymic nude mice.

These results suggest that LC 9018 protected lethally irradiated mice from bone marrow death by accelerating granulopoiesis rather than lymphopoiesis and that the contribution of the activated T lymphocytes to the enhancement of the granulopoiesis was small.

Publication:

Furuse, M., Tsuneoka, K., Uchida, K., Nomoto, K.:J. Radiat. Res., 38, 111-120, 1997.

Fig. 21. Time cause of lineage-specific cell populations of leukocyte in the blood of 6.0- Gy irradiated mice.

3．4． BIO-MEDICALSCIENCE Pathology and Physiology

42．Demonstration of Rat CAR Bacillus Using a Labelled Streptavidin Biotin (LSAB) Method

Satoru Matsushita 1, Akihiro Kawano, Jorge Oros 2, Jose L. Rodriguez 2, Francisco Rodriguez 2, Antonio Fernandez 2 and Tsuneya Matsumoto (1 Institute for Environmental Sciences, Rokkasho, 2 Faculty of Veterinary Science, University of Las Palmas de Gran Canaria, Spain)

Keywords: CAR bacillus, immunohistochemistry, LSAB method, rat

Cilia-associated respiratory (CAR) bacillus is a descriptive term for unclassified, gram- negative, motile, filamentous bacterium that colonizes the respiratory epithelium of laboratory and domestic animals. The infection has been diagnosed by histological, electron microscopical and serological examinations. Immunostaining methods such as indirect immunofluorescence (IF) and avidin-biotin complex (ABC) methods have also been reported. However, these methods require an IF microscope or are less sensitive. In this paper, we describe immunohistochemical detection using an immunoperoxidase technique based on the labelled streptavidin biotin (LSAB) method and 3-amino-9- ethylcarbazole (AEC) as substrate. Specific hyperimmune sera raised in mice against the SMR strain of CAR bacillus isolated from a naturally infected rat were used. Lung tissues from rats experimentally infected with the same strain were used as positive control. For immunohistochemical labelling, the lung sections were dewaxed and immersed in 3% H_2O_2/methanol. The sections were exposed to polyclonal antisera against rat CAR bacillus applied at dilutions ranging from 1:100 to 1:1200, and incubated with a 1:5 dilution of biotinylated antibody to mouse and rabbit IgG (LSAB Kit; Dako), and then incubated with a 1:5 dilution of streptavidin peroxidase complex reagent (LSAB Kit; Dako). The sections were exposed to AEC as substrate. The same procedure was carried out using 3,3'-diaminobenzidine (DAB) as substrate. ABC staining was performed by using the same anti-rat CAR bacillus sera applied at the same dilutions and an ABC staining kit (Vectastain ABC Kit; Vector) using both AEC and DAB as chromogens.

By the LSAB method, strong immunoperoxidase red labelling of bacteria lining the ciliated bronchial epithelium from the CAR bacillus infected rats was clearly detected using the anti-rat CAR bacillus mouse sera at a dilution of 1:800, whereas no immunoreaction was observed in pulmonary ciliated epithelium of non-infected rats when the same sera were used. Background was not evidenced at this dilution using AEC as substrate. However, moderate background was detected using DAB as substrate. Furthermore, the ABC method was less sensitive than the LSAB method.

The results of the present study indicate that this immunohistochemical test using polyclonal antisera can be used to detect CAR bacillus in pneumonic lesions from infected rats and to study the pathogenesis of this disease. The use of AEC as substrate for the immunoreaction was evaluated as being more efficient than DAB because of the background observed using DAB and the strong red colour provided by AEC

facilitating the interpretation of results. It can be concluded that the immunoperoxidase technique based on the LSAB method using AEC as substrate is a very useful method for the detection of CAR bacillus antigen in paraffin-embedded sections.

Publication:

Oros, J., Matsushita, S., Rodriguez, J. L., Rodriguez, F. and Fernandez, A.: J. Vet. Med. Sci., 58, 1219-1221, 1996.

43. Dose and Dose Rate Effectiveness Factors of Radiation-Induced Myeloid Leukemia in C3H Male Mice

Takeshi Furuse, Yuko Noda, Akihiro Shiragai, Hiroshi Otsu and Norikazu Yasuda

Keywords: radiation carcinogenesis, myeloid leukemia, dose rate effect, DDREF, low dose rate

We investigated the induction of myeloid leukemia and other kinds of neoplasias in C3H male mice irradiated at several dose rate levels. We compared the incidence of neoplasias among these groups, and obtained dose and dose rate effectiveness factors (DDREF) for myeloid leukemia.
6026 C3H/He male mice were exposed to whole-body gamma-ray irradiation at or near, 8 weeks of age, and another 507 mice were used for unirradiated control. All mice were maintained for their entire life span and then pathologically examined after their death. Radiation at a high dose-rate of 882mGy/min was delivered from a Cs-137 source of 115 TBq (group H) and at low dose-rates of 0.298mGy/min (group L-A), 0.061mGy/min (group L-B) or 0.016 mGy/min (group L-C) from a Cs-137 source of 0.374 TBq. A medium dose-rate of 95.6mGy/min was delivered from a Cs-137 source of 185 Tbq (groupM: pathological examination incomplete). The mice in group L were irradiated continuously for 22 hours daily up to total doses of 1, 2, 3, 4, 10 Gy over a period of 3 days to 200 days according to the experimental design.
As for the induction of neoplasias, myeloid leukemia, and lung tumors developed significantly more frequently in irradiated groups than in unirradiated groups. The various types of neoplasias included liver tumors, thyroid gland tumors, Harderian gland tumors, adrenal gland tumors, thymic lymphoma and fibrosarcoma in soft tissue. The time distribution of mice dying from myeloid leukemia did not show a difference between groups H and L. The incidence of myeloid leukemia showed a greater increase in the high dose-rate groups than in the low and medium dose-rate groups in the dose range over 2 Gy, but in the lower dose range, the difference between these groups was not clear, except the fact that the 0.25 Gy group H showed a significant increase. These dose effect curves had their highest values on each curve at about 3Gy. Peak values were 23.6, 11.1, 7.3, 7.2, and 6.3 % for groups H, M, L-A, L-B, and L-C, respectively. Incidences in these groups decreased in the upper dose range over 4 Gy, possibly because of the killing effect of irradiation on carcinogenic target cells. The maximum incidence was compared between groups H and L-C to calculate DDREF as 3.7 (23.6/6.3).

44. Effects of Heavy Ion Particle Beam Irradiation to Whole Body on the Bone Metabolism in Rats

Satoshi Fukuda and Haruzo Iida

Keywords: heavy ion particle beam, whole body irradiation, bone histomorphometry, wistar rat, spontaneously hypertensive rat , HIMAC

Effects of low linear energy transfer (LET) heavy ion particle irradiation to the whole body on the bone metabolism of Wistar Mishima (WM) rat as a model of normal bone metabolism and the stroke-prone spontaneously hypertensive (SHRSP) rat as a model of osteoporosis were examined by Heavy Ion Accelerator in Chiba (HIMAC). Female rats of both strains, at the age of 3 months, were irradiated once to the whole body with doses of 1.25 and 2.5 Gy by the heavy ion particle beam (290MeV/u Carbon beam; LET, 42.35KeV/mm). The bones and serum were collected three months after irradiation. In the histomorphometric measurement of the secondary spongiosa area of tibial proximal metaphysis, the osteoid volume in WM rats decreased significantly with increase of dose, and a significant decrease of trabecular separation and increase of osteoid volume in the
1.25 Gy group of SHRSP rats were observed. However, dynamic parameters such as bone mineralizing surface/bone surface and bone formation rate did not significantly change. Also, the values of serum PTH, calcitonin and ionic calcium concentrations, and bone strength and calcium content of the femur did not change. The results indicate that severe osteoporosis might be induced by the low LET heavy ion particle beam irradiation to the whole body in young adult rats.

Publication:
Fukuda, S. and Iida, H.: J. Jpn. Soc. Bone Morphom., 6, 287-291, 1996.

Figure legends

Fig. 1 Histomorphometric values in the proximal metaphysis of tibia in WM rat and SHRSP. Bone volume (BV/TV), trabecular separation (Tb.Sp), osteoid volume (OV/TV), mineralizing surface/bone surface (MS/BS) and bone formation rate (BFR/BV). Values are mean ｱ SE (n = 5)

4 5. Excess Mortality and Temporal Pattern of Manifestation in Mice irradiated with 0.19 Gy Gamma Rays at 7 Days of Age.

Shunsaku Sasaki

Keywords: low-dose radiation, relative risk, cumulative excess mortality, temporal pattern, B6C3F1 mice

In our previous studies it has become evident that mice aged from Lneonatal to puberty periods are most susceptible to life-shorting effect of radiation. Further experimental studies were designed to elucidate the characteristics of long-term effects of exposure of juvenile mice to ionizing radiation including a low-dose range.The present report describes shortening of the mean lifee span, relative risk, cumulative realtive risk and cumulative excess mortality in female B6C3F1 mice irradiated with 0.19 Gy game a rays from 137Cs at 7 days of age. Sample size of the irradiated group was 393 and that of the control group was 885. All the ce were allowed to live through their entire life spans under a specific pathogen free condition. The age-specific mortality was calculated for unoverlapping interval of age, and the cumulative excess mortality was obtained from increase in age-specific mortality.Mean life span of the irradiated group was 832.5±6.4 days, which was significantly shorter than that of the control group (864.8±4.6 days). Shorting of the mean life span, therefore, was 3.7±0.9 % Temporal variations of the relae risk, cumulative relative risk and cumulative excess mortality are summarized in Table 5. It seems important that the relative risk was almost constant. Lifetime relative risk, that is the final value of cumulative relative risk, was 1.30±0.07. The cumulative relative risk did not offer from 1.0 up to 650 days of age; thereafter it began to increase and reached a constant value. Lifetime excess mortality was 0.228±0.005. This result implies that 22.8 % of deaths in the irradiated group were attributable to radiation exposure. Temporal variation of the cumulative excess mortality also indicated that the latent period for manifestation of radiation-induced lethal effects was as 650 days.Results of the present experiment have clearly shown that irradiation with 0.19 Gy gamma rays on mice 7 days old induced late-occuring lethal effects.

Table 5. Relative risk, cumulative risk and cumulative excess mortality in mice irradiated with 0.19 Gy gamma rays at 7 days of age.

Age, days	**Relative risk**	**Cumulative relative risk**	**Cumulative excess mortality**
250-450	2.12±1.93	2.12±1.93	0.004±0.005
450-550	0.90±0.38	1.05±0.24	0.006±0.011
550-650	1.00±0.31	1.02±0.25	0.016±0.017
650-750	1.32±0.21	1.21±0.16	0.041±0.022
750-850	1.41±0.14	1.32±0.10	0.138±0.041
850-950	1.21±0.12	1.28±0.08	0.178±0.041
950-1050	1.41±0.17	1.30±0.07	0.222±0.049
1050-1150	1.09±0.24	1.29±0.07	0.266±0.050
1150-1250	5.25±2.34	1.30±0.07	0.228±0.050

46. Acute Effects of Ionizing Radiation on scid Homozygous, scid Heterozygous and Wild-Type Mice in *in Vivo* and *in Vitro*

Shigeru Kobayashi, Mayumi Nishimura, Yoshiya Shimada, Fumio Suzuki1, Atsuko Matsuoka2, Hiroko Sakamoto2, Makoto Hayashi2, Toshio Sofuni2, Toshihiko Sado and Toshiaki Ogiu
(1 Hiroshima Univ.; 2 NIHS)

Keywords: scid mutation, radiosensitivity, partial dominance, mice, bone marrow cells

Since Fulop and Phillips first reported the hypersensitivity of SCID mice to ionizing radiation, the effects of the scid mutation on repair of DNA double strand breaks have gradually been clarified. Thus the affected mice are known to have defective rejoining of the breaks that occur in the process of V(D)J recombination or are caused by ionizing radiation. However, the relationship of this sensitivity to mutagenicity and carcinogenicity and gene-dosage effect on the scid gene have not been extensively examined. In the present study, acute effects of ionizing radiation on survival of the animal, bone marrow cells and fibroblast cell lines of scid homozygous (SCID), *scid* heterozygous (F1) and wild-type mice with the same C.B-17 genetic background were examined. The sensitivities to ultraviolet light (UV) and various chemicals (bleomycin, mitomycin C, Nmethyl-N'-nitro-N-nitrosoguanidine, methyl methansulfonate, 5-fluorouracil, 6-mercaptopurine, 4-nitroquinoline 1-oxide and potassium bromate) were also investigated. In addition, micronucleus testing of whole body irradiated mice was performed.

In the lethality experiment in animals, all SCID mice died at 5 Gy but survived at 3 Gy, the maximum tolerated dose. C.B-17 mice died at doses of 7 Gy or more, and F1 mice, at doses of 5.5 Gy or more. The LD50(30) was 4.05, 6.5 and 7.2 Gy in SCID, F1 and C.B-17 mice, respectively. The survival days postirradiation were shorter in F1 than in C.B-17 mice: 12.1±1.7 and 17.2±4.7 days with the 7.5 Gy dose, and 10.1±2.8 and 15.2±3.9 with the 8.0 Gy dose, in F1 and C.B-17 mice, respectively. These results indicate that the scid heterozygous mice is more sensitive to ionizing radiation than the wild-type mice.

Survival curves for C.B-17 and F1 bone marrow cells after X-ray irradiation had a shoulder region and decreased exponentially with increase of dose. The D0 values (coefficient of slope) were almost the same; 0.68 and 0.67 Gy for C.B-17 and F1 cells, respectively. In contrast, no shoulder was observed for the dose-response of SCID bone marrow cells and D0 value was 0.46 Gy. The D10 values (10% survival dose) were 3.1, 2.8 and 1.0 Gy for C.B-17, F1 and SCID mice, respectively. These results demonstrated that SCID bone marrow cells were radiosensitive and suggested that F1 cells were more sensitive to X-rays than wild-type bone marrow cells.

On the other hand, the SCID cell lines were about twice as sensitive to ionizing radiation

as C.B-17 and F1 cell lines, while the radiosensitivity of two *scid* heterozygous cell lines was comparable to that of three wild-type C.B-17 cell lines. There were no differences in sensitivity of SCID cell lines to UV light and various chemicals, as compared with wildtype and *scid* heterozygous cell lines *in vitro*. Furthermore, micronucleus test demonstrated no significant correlation among SCID, F1 and C.B-17 mice.
In conclusion, *scid* heterozygous mice were found to be less sensitive than the *scid* homozygous mice but more sensitive to ionizing radiation than wild-type mice not only *in vivo* but also for bone marrow cells *in vitro*, suggesting the partial dominance under both conditions.

Publication:

Kobayashi, S., Nishimura, M., Shimada, Y., Suzuki, F., Matsuoka, A., Sakamoto, H., Hayashi, M., Sofuni, T., Sado, T. and Ogiu, T.: Int. J. Radiat. Biol., 72, 537-545, 1997.

47. Superparamagnetic Iron Oxide Enhanced MRI of Radiatin Induced Liver Injury in Rat

Naoki Morimoto*, Masaaki Ebara*, Hirotoshi Kato, Takayuki Obata, Junichi Fujita, Shigeo Furukawa, Koichi Ando, Tadaaki Miyamoto, Hirohiko Tsuji, and Hiromitsu Saisho* (*Chiba Univ., School of Medicine)

Keywords: MRI, radiaiton induced liver injury, superparamagnetic iron oxide

The detectability of early liver injury induced by irradiation was studied using magnetic resonance imaging (MRI) enhanced with superparamagnetic iron oxide (SPIO), a tissue- specific contrast agent against the reticuloendothelial system (RES) of the liver. In 36 rats, the right side or the left side of the liver was irradiated with a single dose x-ray(0- 10Gy). Non-enhanced and SPIO-enhanced MRI were performed 3 days after irradiation. Speciments were obtained immediately after imaging and stained with Berlin blue to observe the phagocytic function of RES. The irradiated portion of the liver could be visualized with a clear demarcation from the nonirradiated part by SPIO-enhanced MRI as a decrease of negative enhancement reflecting the function of RES ($p<0.05$), whereas this was impossible with non-enhanced MRI. Significant regression was observed as a dose-related change of the signal intensity in the irradiation portion on SPIO-enhanced MR images ($R=0.867$, $p<0.0001$). SPIO-enhanced MRI was reliable for detecting the range and extent of liver injury a few days after even low-dose irradiation, and it may be a useful procedure for verifying the target area in slinical cases of radiation therapy.

3．5．BIO-MEDICAL SCIENCES　Genetics

48．Cryopreservation of Embryo and Spermatozoa for Strain Maintenance of Mice

Masanori Okamoto, Tsuneya Mastumoto, Naomi Nakagata 1 and Hiroshi Suzuki 2 (1 Institute of Medical Science, Univ. of Tokyo, 2 Chugai Pharmaceutical Co., Ltd.)

Keywords: cryopreservation, embryo, mouse, spermatozoa, strain maintenance

This report introduces of reproductive biotechnology mainly an embryo manipulation technique, for the purposes of strain maintenance and management of laboratory animals. We developed a strain maintenance system using the embryo cryopreservation method on inbred mice which were produced in our facilities, besides cleaning of HVJ infected mice and producing of germfree mouse which used embryo transfer technique. We examined strain maintenance of inbred mice by comparing an ultrarapid freezing by vitrification method and a slow freezing method. Slow freezing method suited our purpose and we carried out embryo cryopreservation for about 27 strains of mice produced in our facilities. We used slow freezing with a programmable electrical cooling freezer. We completed the frozen storage of 4,541 embryos. The strain maintenance of transgenic and gene disrupted mice need not always be conserved by freezing an embryo, if the manipulated gene is transmitted to the next generation. Using spermatoza is superior to embryo use, and many samples can be collected easily at once. So we examined the effectiveness of cryopreservation using spermatoza obtained from heterozygous gene disrupted mice of 2 lines. Recently, these mice had been much studied for gene function analysis in our institute. The oocytes collected from wild type mice were inseminated with frozen-thawed spermatozoa. The newborn young which were produced by embryo transfer were analyzed for transmission of the manipulated gene afterwards. As a result, disrupted allele to the newborn young were transmitted, and the difference between expected value of incidence in heterozygous gene disrupted mice and the observed value was not significant for either gene disrupted mice of 2 lines. In mice of 2 lines, the progeny test of mating between heterozygous gene disrupted mice demonstrated that mutant homozygous mice were produced. Our present results revealed that the cryopreservation method of mouse spermatoza can become a technology which is useful as a strain maintenance method for transgenic and gene disrupted mice.

Publications:

1) Okamoto, M. and Mastumoto, T. : Germfree Life and its Ramifications, K.Hashimoto et al.(eds.), XIIth ISG Publishing Committee, 21-24, 1996.
2) Aoki, K., Okamoto, M., Tatsumi, K. and Ishikawa, Y. : Zoological Science, 14, 641-644, 1997.
3) Nakagata, N., Okamoto, M., Ueda, O. and Suzuki, H. : Biol. Reprod., 57, 1050-1055, 1997.
4) Okamoto, M., Nakagata, N., Ueda, O., Kamada, N. and Suzuki, H. : J. Mamm. Ova Res., 15, in press, 1998

49. A Recessive Lethal Mutaion, *tb*, That Bends the Midbrain Region of the Neoutral Tube in the Early Embryo of the Medaka

Yuji Ishikawa

Keywords: neutral tube, development, mutant, brain, medaka, fish

A recessive lethal mutation, *tb (twisted brain)*, in the homozygous embryo of which the neutral tube is twisted, was newly found in the medaka *(Oryzias latipes)*. The mutaion was recovered in the progeny of a male medaka which had been treated with N-ethyl-N- nitrosourea (ENU). The mutation affected morphogenic cell movements (extension and convergence) in the gastrula and neurula stages. In these stages, the embryonic body was shorter and the convergence of the neutral ectodermal cels proceeded more slowly, especially in the prospective midbrain region, in the mutant embryo than in the wild-type embryo. As the neutral rod formed in the mutant embryo, it curved in the midbrain region, usually protruding to the right as viewed from above. Structures lying anterior and posterior to the midbrain region were invariably present and developed fairly normally in the mutant embryo. These results show that there exists a genetically separable component in the developmental process of the formation of a straight and symmetrical neutral tube.

Publication:

Ishikawa Y.: Neurosci. Res. 24, 313-317, 1996.

5 0. Predominance of Mutations with Extensive Deletions in Ataxia Telangiectasia Lymphoblastoid Cells Treated with Ionizing Radiation

Kouichi Tatsumi, Akira Tachibana* , Yuko Houki-Fujimori, Akira Fujimori, Takesi Kato* and Masao Sasaki* (* Radiation Biology Center, Kyoto Univ.)

Keywords: ataxia telangiectasia, deletion, HPRT, APRT, LCL, RFLP

Ataxia telangiectasia(AT) is an autosomal recessive hereditary disease distinguished by a high incidence of lympho-reticular malignancy, cerebellar ataxia, defects in the immune system, and a hypersensitivity to cell killing by ionizing radiation. As far as 6-thioguanine resistant (TGr) marker is concerned, however, EBV-immortalized lymphoblastoid cells from an AT patient are not more mutable by γ-rays than those from normal controls (Tatsumi, K. and Takebe, H., Gann, 75: 1040-1043, 1984).

We examined the molecular nature of the hypoxanthine-guanine phosphoribosyltransferase *(HPRT)* gene in TGr mutant clones from an AT lymphoblastoid cell line, GM2783, by employing the multiplex enzymatic amplification of all exons except for the exon 1. Twelve out of 15(80%) g-ray-induced TGr mutants from GM2783 showed loss of some exons, implying deletions in the *HPRT* gene. Moreover, 8 of these 12 deletion mutant clones lacked all the exons examined (from exon 2 to exon 9). Five mutants had also lost both DXS79 and DXS86, flanking markers of the *HPRT* locus. In contrast, only 38 % (18/48) of γ-ray-induced TGr mutants from a repair-proficient cell line, TK6, showed deletions, and no total deletion of the *HPRT* gene was detected for TK6 cells. Thus, prevailing mutations induced by γ-rays in AT cells seem to be extensive deletions, probably leading to the poor recovery of mutants induced by ionizing radiation at the hemizygous *HPRT* gene from AT cells.

In an attempt to isolate from AT cells a heterozygously inactivated clone at the adenine phosphoribosyltransferase gene on the autosome 16 (*APRT*+/-), AT1-1 cells were treated with 0.5 μM of ICR-191, a frame-shift mutagen, for 24 h. Following this treatment, APRT deficient mutants resistant to 100 μM 2,6-diaminopurine(DAP) were found to be generated at the frequency of 4.9 x 10-6, much higher than expected for a 2-hit event, i.e., *APRT*+/+ _> *APRT*-/- or *APRT*-/o. Southern blot analyses revealed that AT1-1 cells were heterozygous for TaqI *RFLP* at the *APRT* gene, and that all of the 11 mutant clones examined lost either a TaqI-site-(+) or a TaqI-site-(-) allele. When the mutagenic effectiveness of ICR-191(2 x 10-5) to the other *APRT*+/- human lymphoblastoid cells at the same concentration employed was taken into account, these results collectively implied that the AT1-1 cell population had accumulated *APRT* hemizygous cells (*APRT*+/o) at an extremely high frequency prior to the ICR-191 treatment, presumably reflecting an excessive genome instability of AT cells.

5 1．Cell Killing and Mutation Induction by g-rays of Different Dose Rates in Thymic Lymphoma Cells Derived from a SCID Mouse

Ikuko Furuno-Fukushi, Kouichi Tatsumi, Masahiro Muto, Fumiaki Watanabe and Toshiaki Ogiu

Keywords: SCID mouse, g-rays, mutation induction, Hprt, dose-rate effect

SCA1 is a lymphoid cell line derived from a spontaneous thymic lymphoma in a severe combined immunodeficient (SCID) mouse. This cell line was very sensitive to killing by acute γ-rays (30 Gy/h). DNA dependent protein-kinase activity determined with the crude extract of SCA1 cells was negligibly low and so indicated that this tumor cell line retained the mutated phenotype as SCID. Induction of mutation to 6-thioguanine resistance in SCA1 cells was studied after exposure to γ-rays at dose rates of 30 Gy/h, 0.21 Gy/h and 0.0048 Gy/h. A slight increase was observed in cell survival with decreasing dose rate. The magnitude of the dose-rate effects was not as marked as that observed in L5178Y cells. SCA1 cells were hypomutable by γ-rays at dose rates of 30 Gy/h. Mutant fraction increased linearly with increasing dose for all dose rates examined. No significant difference was observed in the mutant fraction versus total dose at the three different dose rates in SCA1 cells. These results suggest that SCID mutation renders mouse cells hypomutable by g-rays and that the repair pathway that is defective in SCID appears to be responsible at least in part for the mutagenesis by DNA double- strand breaks. SCA1 cells are deficient in repair, but not totally for cell killing damage by γ-irradiation.

5 2． Distinct Differences in Intron Structure of Ribosomal Protein Genes between Fission and Budding Yeasts and Its Implications on the Function of Intron

Kazuei Mita, Mitsuoki Morimyo, Etsuko Hongo, Tomoyasu Higashi, Kimihiko Sugaya, Masahiro Ajimura, Yoshie Ishihara, Sun-ichi Sasanuma, Junko Nohata,Terumi Kimura, Yoshiko Koike, Koichi Ito* and Yoshikazu Nakamura* (*Univ. of Tokyo)

Keywords: cDNA catalog, fission yeast, ribosomal protein gene, intron

We have succeeded in summarizing most ribosomal protein genes of *Schizosaccharomyces pombe* ; 52 genes participating in the 40S ribosomal subunit and 66 genes for the 60S subunit were identified by homology search. For nomenclature of ribosomal protein genes, we followed the ribosomal protein classification by SWISS-PLOT, based on amino acid sequence similarity. About a half of the *S. pombe* ribosomal proteins are encoded by two genes, while 60% of the ribosomal protein genes of *Saccharomyces cerevisiae* are duplicated. Amino acid homology of *S. pombe* ribosomal proteins with the *S. cerevisiae* counterparts are observed to be 45 - 90%, which is almost the same as in the comparison with human counterparts. Outstanding difference between ribosomal protein genes of both yeasts is found in features of introns. 67% of *S. cerevisiae* ribosomal protein genes contain introns, which is unusual since it is known that only 2% of non-ribosomal protein genes have intron. Many, but not all, introns of *S. cerevisiae* ribosomal protein genes locate near the initiation codon and 5 cases are on 5' untranslated region. The lengths of the introns are almost all in the range of 300-500 bp. In the case of *S. pombe*, a half of the ribosomal protein genes (33/65 genes) contain intron with 45-300 bp length, whose locations do not concentrate in the vicinity of the initiation codon. It should be noted that the presence of intron in each ribosomal protein gene is not conserved between either yeasts. These facts suggest that the regulation of expression of ribosomal protein genes is not conserved between *S. pombe* and *S. cerevisiae*. Comparative studies of amino acid homology and nucleotide homology of introns between both yeasts show that the event of intron insertion to ribosomal protein genes occurred after separation of species followed by gene duplication.

53. Mutant Reconstitution on RNA Polymerase II Largest Subunit of *Schizosaccharomyces pombe*: Introduction of Mutation Site Related to Abnormal Induction of Sister Chromatid Exchanges

Kimihiko Sugaya, Masahiro Ajimura, Hideo Tsuji, Mitsuoki Morimyo, and Kazuei Mita

Keywords: RNA polymerase II largest subunit, mutant, chromosome instability

For the purpose of confirming a mutation site related to abnormal induction of sister chromatid exchanges in the RNA polymerase II largest subunit (*RpII LS*), and revealing its molecular mechanism, we introduced a mutation into the *Schizosaccharomyces pombe rpb1* gene, encoding *RpII LS*, at the homologous site that we identified in hamster mutant, tsTM4, and investigated the effect. Since the hamster mutant exhibited a decrease of DNA synthesis in the cells arrested in the S phase at the non-permissive temperature, we focussed on analysis of growth, cell cycle and chromosome stability at various temperatures. First, we examined the effects of the mutation on haploid cells.

The mutant showed delayed growth but was not arrested at the non-permissive temperature. With the DNA content of the growing cells at the non-permissive temperature, the accumulation of G1 and/or G0 cells was observed. Tetrad analysis suggested that these phenotypes belonged to the mutation site. Furthermore, for the effects on diploid cell, chromosome instability was found by the loss of intragenic complementation between two alleles of the *ade6* gene. Also concerning the cell cycle, an abnormal fraction containing the intermediate DNA content was observed simultaneously. This accumulated fraction may reflect the cells being in the S phase or the cells having abnormal DNA content caused by chromosome instability. These facts suggest that we could reconstruct the *S. pombe* mutant of *RpII LS* that exhibited a very similar phenotype to the hamster mutant, tsTM4.

5 4．cDNA Catalog of Fission Yeast *Schizosaccharomyces pombe*

Mitsuoki Morimyo, Kazuei Mita, Etsuko Hongo, Tomoyasu Higashi, Kimihiko Sugaya, Masahiro Ajimura, Masatake Yamauchi, Satsuki Tsuji, Woo-Yoon Park, Shunichi Sasanuma, Junko Nohata, Terumi Kimura, Hiromi Inoue and Yoshie Ishihara

Keywords; fission yeast, cDNA catalog

In a study of the structure and functions of the housekeeping genes of eukaryotic cells, we chose *Schizosaccharomyces pombe* as a model organism and analyzed cDNAs of *Schizosaccharomyces pombe (S. pombe). S. pombe* is assumed to have 6,000 genes and the genome size of 14 megabases (Mb) compared to 100,000 genes and 3,000 Mb of humans. In spite of this, it can be used as a model organism for humans, because its genes are similar to human genes which can be normally expressed in yeast, the housekeeping genes are considered to be conserved through eukaryotic evolution and many genes of *S. pombe* have been revealed to have introns which make cDNA sequences essential to determine physical organization of genes from the genomic DNA sequence. Moreover, the functions of *S. pombe* genes are easily known by disrupting them with homologous recombination. The cDNA library containing more than 12,000 clones was prepared by cloning of cDNAs made from polyA+ mRNA into an M13 vector without amplification. In the course of this study, we have developed simple methods to prepare the cDNA library and to determine DNA sequences from both directions by using ss-DNA as the template. All the cDNA clones were sequenced by a single run with a cycle sequencing method using single-stranded DNA as the template. To know the quality of the cDNA library, the cDNA population such as length and number, was examined. The cDNAs longer than 400 bp were 31 % of the population, those ranging from 100 bp to 400 bp were 25%, those below 100 bp were 21 % and mixtures were 23 %. Since we sequenced them all, the cDNA frequency represents the transcription frequency. The most abundant clone was glyceraldehyde-3-phosphate dehydrogenase gene and it appeared over 100 times. This protein is known as a major protein in yeast, often weighing 20 % of total protein. This is in a good accordance with the abundance of the cDNA. Comparison of their DNA sequences with those in a private *S. pombe* cDNA database showed, they were classified into over 2500 independent groups at an average redundancy of 3.2. This covers about 40% of all *S. pombe* genes. Those independent clones were searched in public databases and classified into 1800 newly identified and 900 similar clones, such as those for transcription, translation, helicase, DNA repair, cell cycle, heat shock proteins and various enzymes. As for DNA repair genes, we got 20 genes related to radiation-sensitivity (10 new and 10 known genes). Among 10 new clones, 5 clones have no mammalian homologue genes yet. We could get clues to the human DNA repair genes by using the DNA sequence deduced from conserved AA region between two kinds of yeast genes. Fission yeast is a fantastic model organism and increasing its information about cDNA and genomic DNA sequences, in combination with the complete DNA sequence of budding yeast, will accelerate the cloning and understanding of new structures and functions of human housekeeping genes involving DNA repair and defense against stresses.

Publications:

1) Morimyo, M. and Mita, K.: Nucleic Acids Res. 21, 1679-1680, 1993.

2) Mita, K., Morimyo, M., Hongo, E.: Nucleic Acids Res. 22, 1507-1508, 1994.
3) Morimyo, M., Mita, K., Hongo, E., Higashi, T., Sugaya, K., Ajimura, M.,, Yamauchi, M., Tsuji, S., Park, W-Y., Sasanuma, S., Nohata, J., Kimura, T., Inoue, H., Ishihara, Y.: International Symposium on Biodefence Mechanisms, Chiba, 1996.

55. The Structure and Organization of the Human NPAT Gene

Takashi Imai, Akiyo Nishiyama, Ryoko Shimada, Masashi Sagara, Hiroko Ito, and Tada-aki Hori

Keywords: ataxia telangiectasia, ATM, NPAT, chromosome11q22-23

Ataxia telangiectasia (AT) is an autosomal recessive gene disorder, and ATM, a housekeeping gene, has been identified as the gene responsible for AT. Recently we found that another housekeeping gene, NPAT, is located upstream from ATM on human chromosome 11. The two housekeeping genes are transcribed in opposite directions and share a 0.5 kb 5' flanking sequence. The structure and organization of NPAT were determined by direct sequencing of cosmid clones carrying the gene and by applying the long and accurate (LA)-PCR method to amplify regions encompassing the exon/intron boundaries and all of the exons. The lengths of the introns except intron 1 were determined from the sizes of the LA-PCR products interposed between two consecutive exons. In the case of the smallest introns, 9, 14 and 16, the length of the intron was determined based on analysis of their nucleotide sequences. The exon sequences are identical to those of the corresponding region of the cDNA. The structural organization of NPAT is indicated in Fig. 22. Based on these data, the exons of the NPAT cDNA were concluded to be spread over at least 44 kb. The coding sequence of the NPAT cDNA is composed of 18 exons, ranging in size from 41 to 1653 bp, with the average size being 328 bp. The first methionine codon of the open reading frame is located in exon 1 whereas the stop codon and the poly A additional signal are located in the last exon, exon 18. In accordance with the general GT-AG rule, all of the introns begin with the dinucleotide GT and end with AG, except for a variant donor site with a GC dinucleotide present at the start of intron 17. The junctions at introns 2, 8, 10, 14, and 17 are type 0
(splicing occurring between codons), the junctions at introns 1, 3, 5, 6, 11, 12, 13 and 15 are type 1 (splicing occurring after the first base of the codon), and the junctions at intron 4, 7, 9 and 16 are type 2 (splicing occurring after the second base of the codon). For amplification of all 18 of the exons from genomic DNA, primer sets specific for each intron were designed and PCR amplification conditions which promote amplification of only the desired region were determined.

It has been suggested that AT heterozygotes have an increased risk of developing cancer, especially breast cancer in women. Frequently, loss of heterozygosity (LOH) at loci on 11q22-q24 have been observed in DNA isolated from tumors of the breast, uterine cervix, and colon, perhaps suggesting the location of a tumor suppressor gene in 11q22- q24. For investigation of the role of NPAT in AT and these tumors with allelic loss of 11q22-q24, appropriate primer sequences and PCR conditions for amplification of all the NPAT exons from genomic DNA were determined.

We previously reported that no recombinations are found among Atm, Npat and Acat1(acetoacetyl-CoA thiolase) loci as determined by fine genetic linkage mapping of the mouse AT region. The results of the LA-PCR analysis using NPAT- and ACAT-specific primers and human genomic DNA allowed us to map ACAT 12 kb centromeric to NPAT.

Publication:

Imai, T., Sugawara, T., Nishiyama, A., Shimada, R., Ohki, R., Seki, N., Sagara, M., Ito, H., Yamauchi, M. and Hori, T.: Genomics, 42, 388-392, 1997.

Figure legends

Figure 22. Structure of NPAT. (A) Gene map of regions flanking the ATM locus. Arrowheads show transcriptional direction. The structures of ATM and ACAT are described by Uziel et al. and Kano et al., respectively. // indicates a gap of sequence. (B) Cosmid clones used for sequence analysis. (C) Exon and intron organization of NPAT. Boxes and vertical lines indicate exons. Horizontal lines represent introns. The white box in exon 18 represents the 3' untranslated region. CDK indicates sequences which may code for possible sites for phosphorylation by the CDK/E2F complex and NLS indicates sequences which may code for possible nuclear localization signals.

5 6．Complementary DNA Cloning for a New Member of Phosphatidylinositol Kinase Superfamily

Toshiyuki Saito, Akiko Hayashi, Sumie Kozuma, Naohiko Seki*, Hideshi Ishii, Miki Ohira* and Tada-aki Hori (*Kazusa DNA Research Institute)

Keywards: ataxia telangiectasia, ATM, phosphatidylinositol kinase

The ataxia-telangiectasia responsible gene, ATM, belongs to the so-called ATM family, some members of which have been demonstrated to be a protein kinase though their authentic substrates are yet unidentified. This family includes FRAP, FRP1, DNA-PKcs and ATM proteins that are involved in cell cycle regulation, checkpoint control and maintenance of genome integrity. The family consists of the expanding phosphatidyl inositol-3 (PI3) kinase superfamily with PI3 kinase and PI4 kinase families. Still increasing numbers of the superfamily members and the conserved amino acid stretches shared among them encouraged us to search for other members by utilizing a degenerated oligonucleotide-primed PCR technique. To identify yet unknown superfamily members for better comprehension of the cell cycle regulation and signal transduction systems, we carried out a reverse transcriptase polymerase chain reaction (RT-PCR) experiment with degenerative primers. Cloning and sequencing of a fraction of the RT-PCR products suggested the existence of at least two novel members. One is another member of PI3 kinase family, the other is a new PI4 kinase described in the present study. A cDNA for the latter putative new member for the PI4 kinase family was cloned from an adult human whole brain cDNA library. The predicted protein product was composed of 961 amino acid residues and contained a sequence feature characteristic for lipid/protein kinases. The protein showed an especially striking homology to the only known PI4 kinase, implying that it is another PI4 kinase which consists of a family with the known PI4 kinase. The messenger RNA was ubiquitously expressed in various tissues, suggesting that the gene plays an essential function in cells, while relatively-higher expression was observed in heart, skeletal muscle and testis. The gene was mapped to chromosome 1q21 by fluorescence in situ hybridization.

57. Radiosensitive Phenotype of HeLa Cells Transfected by a Plasmid Vector Expressing Anti-sense *ATM*cDNA

Masatake Yamauchi, Satsuki Tsuji, Minako Terada, Toshiyuki Saito, Takashi Imai, Tada-aki Hori, and Tstsuya Saeki

Key words: ataxia telangiectasia, Radiosensitive, Genome analysis, anti-sense cDNA, transfection

Ataxia telangiectasia (AT) is a human genetic disease that is genetically recessive and manifests cerebellar ataxia and dilation of blood vessels. AT patients also show immunodeiciency, genomic instability, and high incidence of tumours, as well as hypersensitivity to ionizing radiations.

To investigate the AT phenotypes, we first covered the entire region of the major AT locus at 11q22-24, whire the localization of the responsible gene(s) was suggested by genetic linkage analysis, using yeast artificial chromosome (YAC) clones. Cosmid contig covering AT locus was constructed using YAC clones, and the cosmid clones were used to search the DNA regions that can be transcribed. Four independent transcripts were identified by using the CpG island rescue method, including *ATM, NPAT*, and *ACAT*.

ATM gene was a possible candidate responsible for AT phenotype, since point mutations that would give truncated products were identified in *ATM* genes of AT patients. To establish the functional basis of *ATM* gene for the progression of AT phenotype, we introduced a plasmid vector that carried neo gene as a selection marker and 5 portion of

ATM cDNA (6kb) that was integrated to express its antisense strand, into HeLa cells. Transfectant cells were isolated as G418-resistant colonies. While control HeLa cells with plasmid integration but without cDNA insert showed normal radiosensitivity, transfectant of antisense *ATM* cDNA showed wide range of radiosensitivity, ranging from normal level to AT level.

Figure legend

Fig. 23. Hela cell were transfected with pCIneo plasmids with or without anti-sense *ATM* cDNA insert, and G418 resistant clines were isolated. Radiosensitivity of each clone was assayed by colony formation after X ray irradiation. Anti-sense *ATM* cDNA positive transfectants (7,8 and 9) showed hypersensitivity to X ray irradiation, while the radiosensitivity of an anti-sense *ATM*-negative transfectant (12) was not different from wild type HeLa cells. ATA and AATC are cell lines established from AT patients, and showed hypersensitivity to X ray irradiation.

5 8．Molecular Analysis of the Relationship between Radiologically Induced Mutations and Genes Involved in the DNA Precursor Metabolism.

Masatake Yamauchi, Satsuki Tsuji, Toshiyuki Saito, Hideo Tduji, Tetsuya Saeki, Etsuko Hongo, Mitsuoki Morimyo, Kazuei Mita, Masahiko Takahagi, Koh-ichi Tatsumi and Tada-aki Hori

Keywords: purine, pyrimidine, genome analysis, mutagenesis, genetic variation

Despite recent achievements in the analyses of the primary structure of the human genome, functional aspect have yet to be analyzed. Our three year project aimed at establishing the first model system to analyze the functional organization of the DNA precursor metabolic pathway (DPM pathway) at the molecular level, by combining information obtained from biochemical analyses, mutation analyses, and primary structure analyses of the genes involved in the pathway.

To investigate the relationship between the DNA precursor metabolism and the mutagenesis caused by the environmental mutagen at the molecular level, we have started to isolate genes involved in the DNA precursor metabolism of various organisms, human, mouse, avian, and fission yeast. We isolated fourteen human cDNA clones, seven rodent ones, four avian ones, and twelve yeast ones in the first two years. Their nucleotide sequences were determined in parallel with isolation, completely for a full- length cDNA clone of the human *purH* gene, and partially for thirty-six other incomplete- length ones. In the third and final year, full length cDNA clones were isolated for incomplete ones, and their nucleotide sequences were determined. Biological functions of the cDNA clones were examined for human *purH* gene by functional complementation achieved bu introducing the cDNA clones, biological assay are yet to be done.

We have isolated all genes involved in purine and pyrimidine biosynthesis, except for AIR carboxylase, although some genes were left to be isokated for each species. These cDNA clones can be useful materials in examining the relationship between radiologically induces mutations, genomic instability, and polymorphism of genes involved in the DNA precursor metabolism.

5 9．Fine Physical Mapping of a Distamycin A-inducible Fragile Site, FRA8E

Tada-aki Hori, Naohiko Seki*, Miki Ohira*, Toshiyuki Saito, Masatake Yamauchi, Masashi Sagara, Akiko Hayashi, Satsuki Tsuji, Hiroko Ito, and Takashi Imai.(* Kazusa DNA Res. Inst.)

Keywords: distamycin A-inducible fragile site, FRA8E, 8q24.1

Expansion of repeated DNA sequences comprises a unique category of human mutations. Chromosomal fragile sites are known to be particular loci that exhibit chromosomal instability. Five folate-sensitive fragile sites, FRAXA, FRAXE, FRAXF, FRA16A and FRA11B, have been cloned and characterized. In all cases, the mutation leading to the fragile site expression is a new class of mutation (called dynamic mutation) of polymorphic (CCG)n trinucleotide repeat expansion. Recently, a distamycin A-inducible fragile site, FR16B, was shown to be due to a dynamic mutation of a 33pb AT-rich minisatellite repeat.

In our population cytogenetic studies, we have identified five distamycin A-inducible fragile sites, FRA8E, FRA11I, FRA16B, FRA16E, and FRA17A. Among them, the genomic region of FRA8E at 8q24.1 has been shown to include various loci implicated in genomic instability and tumorigenesis. In an attempt to clone the DNA sequence responsible for the FRA8E, we have successively identified a YAC clone, YAC 875F2, which spans the FRA8E locus. This YAC clone is 1000kb in size and covers 7 STS markers between D8S1205 and D8S47. This genomic region has been shown to include breakpoints of various chromosomal rearrangements associated with the Langer-Giedion syndrome (LGS), which is a contiguous gene syndrome, caused by the deletion of both the trichorhinophalangeal syndrome (TRPS1) and hereditary multiple exostoses (EXT1) genes.

To further localize the FRA8E locus in the YAC 875F2, seven P1 clones were isolated from the total human P1 library by PCR-based screening with five DNA markers present in the YAC 875F2 clone. In addition, because the genomic region covered by the YAC clone seems to coincide with the location of the hereditary multiple exostoses (EXT1) gene, we isolated 4 P1 clones that cover both 3'- and 5'-UTRs of the EXT1 gene. Fig. 24 shows relative location of the P1 clones isolated. These P1 clones were labeled with biotin-16- dUTP and were used as FISH probes against the FRA8E-expressed metahase spreads to determine the relative positions of the probes to the FRA8E. The positions of the FISH signals from the two P1 clones, P1-24-11H and P1-46-6B, were found to be localized to the gaps produced by FRA8E expression in most of the cases examined. The FISH signals from the four P1 clones, P1-49-2D, P1-113-5A, P1-71-12D and P1-73-6G, were localized at a region proximal to the FRA8E locus. The other five P1 clones produced hybridization signals at the distal part of the FRA8E expression site. The distribution of FISH signals from each P1 clone generally correlated with its relative position in the physical map shown in Fig. 24. Because the P1-24-11H contained the locus of D8S527, it seems likely that the FRA8E locus lies within a 400kb genomic region between D8S1010 and D8S522 loci, which includes the 5'-region of the EXT1 gene.

Publication:

Hori, T., Seki, N., Ohira, M., Saito, T., Yamauchi, M., Sagara, M., Hayashi, A., Tsuji, S., Ito, H., and Imai, T.:

Cancer Genet. Cytogenet., 99, 1-11, 1997.

Figure legend:

Fig. 24. Localization of P1 clones on an STS-content map of YAC clones spanning the FRA8E locus. Relative position of the EXT1 gene and the direction of its transcription, including the first intron of 250kb length, are noted.

60. Studies on the Growth Failure and Premature Death of *xpg*-deficient Mice.

Tadahiro Shiomi, Yoshi-nobu Harada, Naoko Shiomi and Manabu Koike

Keywords: group G xeroderma pigmentosum, xpg, mouse model, growth failure, premature death

Studies have been carried out to find out the cause of the post-natal growth failure and premature death of *xpg*-deficient mice. The body size of the mutant homozygotes at birth appeared to be almost identical to those of the normal littermates, i.e. the wild type and heterozygote pups. However, at 5 days *post partum*, the mutant homozygotes were easely distinguished from the normal littermates because of their small body sizes. After around 13 days *post partum*, most mutant homozygotes became thin, apparently emaciated, and weak, and their activity was relatively low.

At first, we observed the -/- pups very closely. However, we were unable to detect any obvious physical changes and abnormal behaviors involving suckling. The mutant homozygotes seemed to cling normally to the teats of their dams as did the normal mice. However, the normal littermates might have interefered with the mutant homozygotes' clinging to the mother. Thus, to eliminate that possibility, we removed all the normal size pups from the nest, and kept only the small pups (presumably the mutant homozygotes) at 10 days *post partum* together with their mother. They appeared to be suckled normally. Nonetheless, all the small pups, i. e. the mutant homozygotes, died off before weaning, indicating their genetical disability for surviving.

Next, we carried out histological and anatomical analyses for several organs of *xpg* mutant mice at 0, 5, 16 and 21 days *post partum*. Between the mutant homozygotes and the normal littermates at 0 and 5 days *post partum*, an obvious difference was found only in the small intestines. At 0 day, the small intestines of the mutant homozygotes were apparently smaller in diameter than those of the wild-type mice and heterozygotes. At 5 days, very immature small intestines were observed in the mutant homozygotes.

The number of villi was much fewer than those of the wild type and heterozygotes. Furthermore, the villi of the small intestines in the mutant homozygotes were narrower in diameter and shorter in length. Obvious defects have not yet observed in other organs examined at 0 and 5 days *post partum*.

However, in the case of the mutant homozygotes at 16 and 21 days *post partum*, abnormalities were observed not only in the small intestines, but also in other various organs . As was observed at 0 and 5 days *post partum*, the small intestines of the mutant homozygotes also looked immature, and the number of the villi was fewer than those of the wild type and heterozygotes. The volumes of the intestines and the stomachs in the mutant homozygous mice were relatively small, and many gas bubbles were observed inside the intestines. The weights of the intestines and stomachs of the mutant homozygotes were significantly lighter than those of the wild type and heterozygous mice. The spleens were very small in all the mutant homozygous mice. In particular, the splenic white pulps were atrophied. In the mutant homozygotes at 16 days, the average value of a spleen weight per body weight was about 28% of that in the normal littermates. Miniaturization of their muscle fibers was observed in cardiac and skeletal muscles.

Liver cells in mutant homozygous mice were remarkably smaller than those in the heterozygous mice. These results strongly suggest that abnormal formation of the small intestines causes insufficient digestion and ingestion of milk, resulting in the severe starvation atrophy. This is the most likely cause of the post-natal growth failure and premature death of the mutant homozygotes.

6 1. Subcellular Localization and Expression of the Human DNA Double-Strand Break Repair Genes Ku p70, Ku p80/XRCC5, and DNA-PKcs

Manabu Koike, Yosh-nobu Harada, Naoko Shiomi and Tadahiro Shiomi

Keywords: Ku p70, Ku p80/XRCC5, DNA-PKcs, Northern analysis, subcellular localization

DNA damages induced by ionizing radiation result in measurable endpoints such as cell death, mutation and cell transformation. In particular, double-strand breaks in DNA due to radiation could induce the lethal effect. The DNA-dependent protein kinase (DNA-PK) complex is a nuclear serine/threonine protein kinase composed of at least three components, Ku p70, Ku p80/XRCC5, and a catalytic subunit (DNA-PKcs). Though a large body of evidence suggests that DNA-PK is involved in DNA double-strand break repair and V(D)J recombination, its physiological function is still unclear.

We have determined chromosomal location of the mouse Ku p70 and Ku p80/XRCC5 genes by in situ hybridization: the Ku p70 gene was localized to mouse chromosome 15 and rat chromosome 7; and the Ku p80/XRCC5 gene was localized to mouse chromosome 1 and rat chromosome 9. Both genes were mapped in a region of conserved linkage homology among the three species, i.e. the mouse, rat and human. These results suggest that these genes originate from a common genetic linkage in mammalian evolution. To determined the size and tissue transcription specificity of the mouse Ku p70 and Ku p80/XRCC5 mRNA, Northern blot analysis was carried out with six mouse tissues. Each tissue expressed one species of the Ku p70 gene transcript with 2.4 kb and one species of the Ku p80/XRCC5 gene transcript with 2.6 kb. In the latter case, however, the brain showed two sizes of transcript, 2.6 and 2.9 kb.

To investigate further the biological significance of these genes, we determined the tissue transcription specificity of the human Ku p70 and Ku p80/XRCC5 mRNA in sixteen human tissues by Northern blot analysis. Each tissue expressed one spicies of the Ku p70 gene transcript and two species of Ku p80/XRCC5 gene transcript. And then we examined expression of Ku p70, Ku p80/XRCC5 and DNA-PKcs proteins in various types of human cells (HeLa and normal diploid fibroblasts TIG-3 and so on). These proteins were expressed in all the cells examined at nearly the same level. We also examined subcellular distribution of these proteins under a fluorecent microscope using antibodies specific to these proteins. In interphase cells Ku p70 and Ku p80/XRCC5 proteins were localized in the nuclei, while DNA-PKcs protein was localized both in the nuclei and cytoplasm (mainly in the nuclei). In mitotic cells these three proteins diffused into cytoplasm and no apparent staining was observed at the interior of chromosomes.

Publications:

1) Koike, M., Matsuda, Y., Mimori, T., Harada, Y.-N., Shiomi N. and Shiomi, T.: Genomics, 38, 38-44, 1996,
2) Koike, M., Ishino, K., Kohno., Y., Tachikawa, T., Kartasova, T., Kuroki, T. and Huh, N.: Cancer Letters, 108, 185-193, 1996.

4.CLINICAL RESEARCH

6 2． Clinical Study for Proton Beam Therapy

Takashi Nakano, Siuroku Morita, Shigeo Furukawa, Tatsuaki Kanai, Kouichi Shibayama, Takayoshi Ishii, Takeshi Hiraoka, Atsuko Ishikawa, Atsuro Terahara, Tatsuya Ohono and Hirohiko Tsujii.

Keywords: proton beam therapy, ocular melanoma

High energy proton beams have been regarded as one of most attractive particles for use in radiotherapy. These beams have a characteristic dose distribution which realizes a sharp distal fall-off depending on their energy and sharp side edge. Proton beam therapy for ocular melanoma has been undertaken from 1985. The NIRS cyclotron generates 70MeV protons, which penetrate tissue to a maximum of approximately 37 mm. In 1988, a new vertical beam port was installed and patients were treated while in a sitting posture. In addition, a CT image-based, computerized treatment planning system was developed for the proton beam treatment. In order to get sharp beam quality and accurate treatment setting with quite good responsibility and reproducibility, the proton beam port was specialized for ocular melanoma and rather small lesions at superficial sites of the body, and treatment systems including patient head setting, eye gazing system, and x-ray verification system have been developed. By 1996, 55 ocular melanomas had been treated with protons. In 1996, the treatment protocol was changed. The patients were treated with 5 fractions over 1.5 weeks with a total dose of 60 Gy for small tumors and 70Gy for medium and large tumors. A new version of the treatment system was developed and utilized for the treatment planning, which substantially decreased planning burden.

This year 8 patients with ocular melanoma were treated. Of the 55 patients treated through 1996, 54 were treated in radical intent. The follow-up periods ranged from 6 months to 11 years. Twelve patients had small to medium tumors and 42 had large tumors. Especially, 60 % of them were located contiguous to or involved in the optic disk or macula which are critical organs relating to complications.

Fig. 25 shows cumulative local control and survival rates. The 5 and 10 year survival rates were 94.5% and 89.1 5 , respectively. Both 5 and 10 year local control rates were 80.9%. Five patients had recurrence, and metastasis developed in 3 patients. In the majority of the patients, the tumor regressed after the first six months, and continuous regression has been observed in most tumors at a slower rate. The 5 year cumulative retention rate of the involved eye after treatment was 69 %. Severe retinitis consisting of retinal bleeding and detachment of retina developed in 5 patients. Eleven patients lost their vision . These results suggested that proton beam therapy for ocular melanoma is one of the better treatment modalities.

6 3． Patient Immobilization and Setup Reproducibility in Heavy Ion Treatment.

Tadashi Kamada, Jun-Etsu Mizoe, Yoshisuke Matsuoka, Takashi Nakano, Tadaaki Miyamoto, Hirohiko Tsujii

Keywords: immobilization, positioning, reproducibility, heavy ion treatment

The use of high-LET charged particles in clinical radiotherapy is expected to yield a better local control in a deeply seated tumor and/or a radio resistant tumor because of their spatial dose distributions and biological properties. The utilization of these advantages of the high-LET charged particles, patient immobilization and positioning have been considered as major issues. Unconventional treatment setups of fixed beam lines of these particles make it difficult to establish a reliable immobilization method and to maintain the quality of the beam delivery for the entire treatment session. To overcome these problems, we developed an original patient immobilization method. The purpose of this study was to establish a reliable patient immobilization and to evaluate its efficacy in heavy ion treatment.

An individual thermoplastic shell combined with an original universal head rest or cradle was made and employed as the immobilization method. Original treatment couches suitable for the immobilization technique were also designed. An on-line positioning system using orthogonal digital X-ray imaging equipment has been employed for patient treatment positioning since the beginning of the HIMAC project. The setup reproducibility in each treatment session was evaluated by this system. For the positioning, orthogonal DRRs made from multiple CT images for the 3 D treatment planning were prepared as simulator films and transferred to the on-line positioning system. The DRRs for positioning were compared with corresponding orthogonal digital X-ray images of simulating carbon beams at the initial cold setups(rehearsal). The positioning error was corrected by the on-line positioning system at the rehearsal and then corrected orthogonal X-ray images were stored in the on-line positioning system as the standard positioning images. In every treatment session, the positioning error was measured and corrected by the system.

During the period of January 1996 through January 1997, 2047 setups of the carbon beam treatment for 111 patients were measured and analyzed. The mean setup displacements(mm) from the isocenter in the medio-lateral, the postero-anterior, and the cranio-caudal direction were 0.238(SD 2.510), 0.590(SD 2.385) and -0.147(SD 4.513) respectively. And 95% of the treatment setup displacements (deviation from the isocenter) were within 5 mm in the medio-lateral, the postrior-anterior direction and within 10 mm in the cranio -caudal direction with our original immobilization system. The actual treatments were given after the correction of these deviations.

An individual thermoplastic shell combined with original universal head rests or cradles has an acceptable reproducibility for use with heavy ion patients in their treatment
sessions. However we recognized the need for further improvement of the reproducibility particularly in the cranio-caudal direction of the thorax, abdomen, and pelvic treatments.

6 4.Clinical Assessment of Tumor/ Tissue Responses of Heavy Ions by Radiobiological Basis and Volume Effect for Treatment Optimization

Takashi Nakano, Atsuro Terahara, Shinitiro Sato, Atsuko Abe, Suho Sakata, Isamu Hayata, and Masako Minamihisamatsu.

Keyword : heavy ion therapy, treatment,cervical cancer

Heavy ion radiation response to normal tissue and tumors of patients with various tumors have been analyzed to optimize heavy ion treatment. Heavy ion beams create a characteristic Bragg,s dose distribution when accelerated enough to penetrate tissues. Moreover, the beams have a 2 to 3 times stronger biological effect of cell killing than conventional x-rays due to the high LET beam character. The physical and biological characteristics of the heavy particle therapy realize superior dose accumulation to target tissues / tumors, while sparing irradiation of surrounding normal tissues This is expected to more efficiently control radiation resistant tumors. However, the assessment of a biological effect with incorporation of a volume effect, which is very important for estimation of the effects in inhomogeneously irradiated tissues, remains to be investigated. Then the following studies were undertaken:

1) Comparative dose distribution analyzing system for treatment of cervical cancer patients.
2) Acute response analysis for cervical cancer patients treated with carbon beam therapy.
3) Chromosomal aberration analysis for cervical cancer treated with carbon beam therapy.

Study1) Comparative dose distribution analyzing system for treatment of cervical cancer patients.In order to point out differences or superiority of dose distribution between heavy ion therapy and conventional treatment including brachytherapy, it is necessary to make an original dose distribution analyzing system of brachytherapy based on CT or MR images.The computerized analyzing system realized the dose distribution of brachytherapy on both CT images and MRI, as well as on orthogonal X-ray images. In addition, DVH analysis of specific target or organs is possible. However, the system requires substantial further development for clinical use.

Study2) Acute response analysis for cervical cancer patients treated with carbon beam therapy.
We introduced Dose Volume Histogram Analysis for analyzing tumor/tissue radiation effect. First, we studied acute response of abdominal organs of the patients with very advanced cervical cancer when treated with carbon ion beam therapy. Then, the degree of the acute reaction and incidence of the reaction were compared to those of conventional treatment. The acute response of intestine was analyzed according to tha RTOG scoring system as shown in Fig.26. There are large differences in acute radiation response of intestine between the carbon beamtherapy and conventional treatment. The acute responses including increased bowel movements and diarrhea were significantly weaker than for the conventional treatment. DVH analysis of small intestine area of both the carbon and photon treatment indicated that the carbon beam treatment realized superior dose distribution in which the small intestine

was significantly less irradiated by the beams compared with photons. The superior dose distribution may be the main reason for the weaker acute bowel reaction.

Study3) Chromosomal aberration analysis for patients with cervical cancer treated with carbon beam therapy.Chromosomal aberration of lymphocytes of the patients with cervical cancer treated with carbon beam therapy and conventional x-ray treatment were comparatively analyzed.The numbers of the patients were 18 for conventional treatment and 8 for carbon beam therapy. The mean incidences of the chromosomal aberration were 49.9 % for conventional treatment and 59.5 % for carbon beam treatment. The RBE of chromosomal aberration was approximately 3, which supported the fact that the RBE of the carbon beams used for cancer treatment was 3 .

Publication:

Terahara A., Nakano T. Ishikawa, A., Morita, S., Tsujii, H.: Int. J. Radiat. Oncol. Biol. Phys. 35, 549-554,1996.

6 5．Research and Development on Cancer Treatment Recording System in HIMAC Radiotherapy

Shinichiro Sato and Kenjiro Fukuhisa

Keywords: HIMAC,medical record management

This year, "Research and Development on Cancer Treatment Recording System in HIMAC Radiotherapy" was promoted by upgrading the PACS-like system which has been operated since 1994. In the beginning, this system just accepted (registered) and provided image data. Research activity this year enabled this system to accept value- added data to primarily registered image data. This means the system can be applied to medical recording. This activity will now be terminated and become more generalized research on medical record management.

6 6.Three Dimensional Verification System for Heavy Charged Particle Therapy

Jun -etsu Mizoe, Atsuroh Terahara, Hirohiko Tsujii, Masahiro Endo, Shin -ichi Minohara, Shigeo Furukawa, Masayoshi Sunaoka, Takayoshi Ishii, Takashi Nakano, Tadashi Kamada, Yoshisuke Matsuoka and Hirotoshi Kato.

Key words: 3D verification system, charged particle therapy Introduction

Physical characteristics of heavy charged particles, having a Bragg peak in their depth-dose profile, require more a precise verification system be used than in conventional radiotherapy. In particular, the recognition of organs which will be involved in the target volume or excluded from it is an important matter in daily verification because of the strong effectiveness of the high LET radiotherapy of charged particles. A three dimensional CT verification system will provide more precise and visible information on soft tissue organs than a conventional verification system by X-ray images.

CT machines have been installed in A and B treatment rooms already, and horizontal CT machine was set in room C at the end of 1997. Treatment coach was shared with the examination bed of the CT and a chair system for a sitting position treatment was prepared in room C. New software utilities will involve realtime dose calculation of heavy charged particle radiotherapy on CT images and will provide shift distance of the treatment couch to a reference position.

For the verification time using CT, dose distribution will be demonstrated on CT images with the status of initial positional setting. The position of the tumor and surrounding normal tissues will be verified by the relation of the organs and dose. When positional revision is needed, the shift distance calculated from CT images will be demonstrated and the treatment coach will be moved automatically.

To verify the positioning more actually, DVHs(dose volume histograms) of the target volume and surrounding organs will be calculated and utilized for evaluation of the target volume dose.

Routine use of the CT verification system in the radiotherapy will provide more precise positional information than a conventional X-ray system dose. Particularly imaging of soft tissue organs three dimensionally will deliver result which accurately reflect radiation dose to the target volume and normal organs.

6 7． Planning System of Heavy Charged Particle using MR Images

Jun -etsu Mizoe, Masahiro Endo, Hiroko Ito, Tadashi Kamada, Yoshisuke Matsuoka and Masahisa Koga

Key words: image fusion, MRI, dose planning system, heavy charged particle

MR imaging provides a lot of important information about a tumor and the surrounding normal organs in oncologic imaging. To utilize MR imaging directly for radiotherapy, an MR image fusion system with planning CT images has been developed for heavy charged particle radiotherapy.

For diagnostic MR images, three MR directions were utilized fro image fusion, trans-axial, coronal and sagittal. To translate the contours delineated on MR images to planning CT images, a three-directional image matching system was introduced. Adjustment of the positional difference between MRI and CT images is done on each image direction separately and 3 or 4 corrections produce a final matching of both images.

Dose calculation using translated contour data is displayed on the final distribution plot of the CT images. Initial results of simulations using HIMAC-patients' data showed that there was some discrepancy between translated contours and reference contours used for actual HIMAC- radiotherapy. Slice thickness and slice space of MR images were considered as one cause of the discrepancy, which included non-visible reference anatomy on both images. After the introduction of the three directional image matching system, most of the discrepancy between the two contours disappeared.

Direct utilization of MR images for the treatment planning will provide a lot of diagnostic information of high quality, specially in cases of brain and head & neck cancers. The planning system will provide improved graphic functions of it's computer and reliable management of the network system connecting necessary instruments.

6 8. Clinical Study of Respiration-gated Irradiation in Heavy Ion Therapy

Yoshisuke Matsuoka, JunEtsu Mizoe, Tadashi Kamada, Yasuhiro Osaka, Hirotoshi Katou, Toshihiro Aoyagi, Tadaaki Miyamoto, Sin-ichi Minohara, and Hirohiko Tsujii.

Keywords: heavy ion therapy, respiratory movement, gated irradiation

In order to achieve excellent radiation dose concentration onto thoraco-abdominal tumors and spare normal tissue surrounding the tumors in heavy ion therapy, respiration-related movement should be compensated for. Consequently, a respiration-gated irradiation system (REGIS) was introduced into HIMAC in June 1996. Up to January 1997, 36 lesions consisting of 21 lung tumors, 13 liver tumors, and 2 mediastinal tumors were irradiated using REGIS. In treatment with REGIS a sensor emitting infrared rays is attached to the thoracic or abdominal wall to measure respiratory movement. A camera senses these rays to detect sensor locations and data are forwarded to a computer system. A curve representing respiratory cycles is displayed, and a trigger level is set on it which is part of a respiratory cycle(approximately a fourth of the expiratory phase side) when beams can be delivered. For treatment planning, patients were scanned with the same positioning and immobilization at treatment using REGIS, and longitudinally well-ordered CT images could be obtained. The CT images were utilized for three-dimensional treatment planning, and 5 to 10 mm safety margins were usually added to tumor contours. Respiration-related movements of a thoraco-abdominal tumor were measured making a comparison between images of inspiratory CT and expiratory CT or using X-ray fluoroscopy. Studied were 33 lung tumors and 17 liver tumors. Average movements of the cranio-caudal direction were 7.4 mm (0-30) for lung tumor and 15.6 mm (0-25) for liver tumor. Lung tumors locating in upper lobe, invading the thoracic wall, and being near the hilar region had 0-3 mm movements, while, those located in the middle or lower lobe of the lung field moved 14.8 mm (0-30). Tumor movements of 3 lung tumors and 5 liver tumors are measured within the gated phase using video images taken at positioning verification. Their longitudinal movements ranged approximately from 3 mm to 5 mm, and the values were considered to be useful for drawing target contours. In order to study the increase of irradiated volumes by respiration, planning target volumes (PTVs) and clinical target volumes (CTVs) of 8 lung cancers which were moved by respiration were compared. PTVs were reconstructed with inspiratory and expiratory CT images, and CTVs were determined with expiratory CT images. Average CTVs and PTVs were 24.0 ml (3.9-55.9), and 61.3 ml (24.9-92.2), respectively. The average ratio of PTV and CTV was 3.2 (1.6-6.4). Nominal DVHs of the liver with 5 cm diameter tumor and 2 cm longitudinal respiratory movement were analyzed. Results showed that dose to the liver was decreased when irradiated using REGIS, e.g. 90% dose volumes with or without REGIS were 150 ml and 253 ml, respectively, and 50% dose volumes with or without REGIS were 244 ml and 389 ml, respectively. It was concluded that respiration-gated irradiation is useful for heavy ion radiotherapy of thoracic and abdominal lesions.

69. Clinical Study on the Evaluation Pulmonary Damage by Heavy Particle Radiotherapy

Shizuo. Hasegawa, Toshiaki. Homma, Masaki..Inoue, Itaru. Ohtsu, Shinichirou. Tomioka, Masaaki. Hagiya, Kenji. Kawakami, Yutaka. Mori, Masaru.
Takamura,Tadaaki. Miyamoto, Hisayuki. Aoyagi, Susumu. Kandatsu, Kyousan. Yoshikawa, Hiroko. Ito, Kazumasa. Kumagaya and Hiroko. Moriya

Keywords: lung cancer, carbon beam, pulmonary function, DVH, perfusion scan, ventilation scan

A new research project to evaluate pulmonary damage by heavy particle radiotherapy started from Sept.1996. To the present, more than 30 patients with non-small cell lung cancer (NSCLC) at stage I and stage ⅢA had been treated with graded dose of a carbon beam. The morbidity scoring system for early effects (RTOG) and late effects (RTOG/EORTC) was sued to evaluate radiation injuries. Acute radiation puneumonia at grade 3 occured for two patients, at grade 2 for one and at grade 1 for one. The other 25 patients had no pulmonary damage symptomatically (grade 0). Late radiation effect on lung showed only fibrotic changes on image films for all patients (grade 1). However, such a score system is not neccesarily satisfactoryed for the evaluation of pulmonary damage. The purpose of this study is to develop a new system with which the lung damage by radiation is accessed in a quantitative way.

Results

1) the pulmonary function for 30 patients was surveyed before and after irradiation in terms of lung function test and blood gas analysis. There were no statistical changes of these parameters, suggesting that the damage to the lung by the carbon beam was too little to be detected by the ordinary pulmanary function test.
2) Lung fibrotisis with a sharp margin appeared on the restricted target area at least 3-4 months after irradiation but did not expand to non-irradiated areas. The influence of this damage on the whole lung has been studied as follows: irradiated lung volume is estimated on the CT image by the DVH method and compared with the morphlogical changes which eventually appeares on CT, and perfusion and ventilation scan images after irradiation.These studies are on going.

70. Clinico-pathological and Molecular Biological Analyses of Heavy Ion Radiation Effect

Takashi Nakano, Tatsuya Ohono, Yuzuru Niibe, Atsuko Abe, Nobuyuki Miyahara, Keiko Higuchi, Hideo Niibe, Kuniyuki Oka

Keyword: heavy ion radiotherapy, cervical cancer, biological effect

Pathological and molecular biological analyses of heavy ion radiation effect of cervical cancer were undertaken. The beams have a 2 to 3 times stronger biological effect of cell killing than conventional x-rays because of the high LET beam character. Therefore, carbon beam therapy is expected to efficiently control radiation resistant tumors.

However, assessment of the biological effect is remain to be investigated. In order to estimate biological characteristics of the carbon beams, establishment of radiobiological scale is essential for comparison of the radiobiological effects between carbon and photon treatments. Hence, the following subjects were studied.

1.inico-pathological analysis on the regression of cervical cancer during heavy ion radiotherapy: carbon beams cause faster radiation response of cervical cancer than X-rays

The purpose of this study is to define differences of early radiation response of tumors between a carbon beam and x-rays. Eleven patients who had been irradiated by carbon beams in the phase I/II study from April 1995 to June 1996 were examined. We enrolled 22 comparative patients who had received conventional photon therapy for uterine cervical cancer of stages III, IV. The intensities of carbon and x-ray radiations were considered the same until 3 weeks. Biopsy specimens were taken from the tumor before irradiation, six hours after irradiation, 1 and 3 weeks after irradiation and at the end of irradiation treatment series. We compare the following points between carbon and x-ray radiations: (1) reduction of the tumor size as calculated with a CT image; (2) histological response; (3) the frequency of radiation-induced apoptosis detected by the TUNEL method; (4) transition of cell cycle related to proteins p53 p21 and Ki-67 (expression stained immunohistochemically). The mean reduction rates of the tumor size calculated with a CT image 3 weeks after the initial radiations of x-rays and carbon were 22 % and 33%, respectively. At the end of the irradiation treatment series, the mean reduction rates of x-rays and carbon were 51% and 54%, respectively. The frequencies of histologically good response for carbon and x-rays were 14% and 50% at 1 week and 48% and 90% at 3 weeks, respectively. This morphological comparison showed that carbon irradiation caused faster morphological response than photons. The frequencies of radiation-induced apoptosis in carbon and x-ray irradiations were 0.22% and 0.12% before and 1.20% and 0.56% 1 week afters, respectively. The radiation-induced apoptosis similarly changed between carbon and x-ray treatments 1 week after the initial radiation. The frequencies of p53 in carbon and x-rays treatments were 7% and 4% before irradiation and 14% and 9% at 1 week, respectively. The frequencies of p21 in carbon and x-rays treatments were 9% and 12% before irradiation and 18% and 22% at 1 week, respectively. The frequencies of Ki-67 growth fraction in carbon and x-ray treatments were 37% and 41% before

irradiation and 55% and 59% at 1 week, respectively. There were no significant differences between carbon and x-ray therapies on p53, p21, Ki-67 growth fraction. The radiation effect appears faster for carbon than for x- rays on the CT image and histological features. The difference in radiation response is not considered to derived from cell death by apoptosis, but rather to other factors such as faster mitotic death or absorption of necrosis.

2.histochemical prognostic factors in radiation therapy of cervical cancer

Immunohistochemical study was performed on 64 cervical cancer patients treated with radiation therapy. Prognosis was analyzed by c-erbB-2 oncoprotein expression(CerbB), growth fraction determined with Ki-67 immunohistochemistry(Ki-GF) and the mitotic index of proliferating cell population(pMI).The 5-year survival rates for the Ki-GF of 33% or greater were significantly better than those below 33% (87.5% vs 44.7%, $P<0.01$). The difference was due to difference in recurrence rates. The 5-year survival rates for patients with pMI of 3.5% or greater were significantly lower than those below 3.5%(0% vs 81.8%, $P< 0.001$). The difference was due also to difference in recurrence rates. CerbB-OE was observed on the cell membrane of cancer cells. Positivity of CerbB, which was 42.4% in total, increased significantly with stage progression. Mean Ki-GF and pMI were 36% and 2.5% in total, respectively. Mean Ki-GF for CerbB(+) patients was 26.2%, significantly lower than the 38.3% for CerbB(-) patients ($P<0.01$). The mean pMI for CerbB(+) patients was 3.70%, significantly higher than the 2.00% of CerbB(-) patients ($P<0.05$). The correlation between CerbB and Ki-GF and pMI suggests that CerbB oncoprotein may play an important role in cell proliferation status of cancer of the uterine cervix. The 5-year survival rates of CerbB(+) and CerbB(-) patients were 45.1% and 75.6%, respectively, indicating that CerbB(+) patients showed significantly poorer survival than CerbB(-) ones ($P<0.01$). The difference in survival was mainly due to local recurrence rather than distant metastasis.The Ki-GF, pMI and CerbB-OE are suggested as significant predictors for local control as well as long term survival. Additionally, these predictors are hopeful as determinants for selecting optimal therapeutic modalities.

Publications:

1) Nakano,T., Oka,K., Hanba,K., Morita,S.: Clin. Immunol. and Immunopathol.79, 79- 86,1996.
2) Oka, K., Nakano,T., Hoshi,T.: Cancer 77,2280-2285,1996.
3) Nakano, T. Oka K, Taniguchi Y.: Cancer Res. 56,2771-2775,1996. Oka, K., Nakano,T., Hoshi,T.: Cancer 77,2280-2285,1996.

Nakano, T. Oka K, Taniguchi Y. Manganese superoxide dismutase expression correlates with p53 status and local recurrence of cervical carcinoma treated with radiation therapy. Cancer Res. 56,2771-2775,1996.

7 1． Carbon Ion Radiotherapy for Lung Cancer

Tadaaki Miyamoto, Hisayuki Aoyagi,Yousuke Matsuoka,Hirohiko Tujii 1and Yutaka Yamaguchi

Keywoards:lung cancer, clinical trial,carbon beam, HIMAC

In our institute, the construction of the heavy ion medical accelerator in Chiba(HIMAC) was completed in October, 1994. The following year , we started to treat head & neck cancer as a pilot study using a carbon beam. Following confirmation of the expected reaction of skin and mucosa membrane, we opened the protocol for lung cancer entitled "A phase I/II and II clinical trials of heavy iron particle radiotherapy for non-small cell carcinoma of the lung" . Since then,crabon beam, possessing well-balanced dual actions on cancer(efficient dose-localization and high RBE) was given to 38 inoperable patients with stage I and 5 patients with stage II A as preoperative radiotherapy. The dose was raised from 59.4GyE , 64.5GyE,72GyE,79.6GyG,86.5GyE and 94.5 GyE by 10% increase. The recurrence rate against time after therapy (Kaplan-Meyer method) is shown in Fig
27. Five patients(50%) given 59.4GyE,64.5GyE,72GyE, more than 80GyE showed 60%,50% 30% and 0%, respectively.　This appeared to be dose-sependent. On the other hand, two of 43 patients(4.6%) showed acute radiation pneumonia which was　treated with steroid hormone. However, the late effect of the lung was weakenend with slight radiographic changes and without any manifestations. Now that heavy ion particle radiotherapy for lung cancer has been shown to be safe,we intend to attain tumor control comparable with that of surgery.

Publication:

Miyamoto T., Aoyagi,H., Tujii, H., and Yamagichi, Y.,: J.Jan. Surg.Soc., 98, 60-67,1997.

72. Phase Ⅱ Study of Combination Therapy of Radiation and Local Administration of OK-432 for Esophageal Cancer

Minoru Mukai and Shinroku Morita.

Keywords: esophageal cancer, combination therapy, radiation, OK-432

From 1993 to 1996, 38 patients with T1-4NxM0 (UICC, 1987)entered the phase Ⅱ study of combination therapy of radiation and local administration of OK-432. There were 32 males and 6 females. The average age was 64 years. The average tumor length was 7.9 cm. There were 7 T1, 12 T2-3, and 19 T4 patients. OK-432, 0.5 mg, was administered endoscopically around the cancerous lesion at the beginning of radiotherapy and the same dose of OK-432 was given in the same manner 2 weeks later. X-ray irradiation was given at a daily dose of 1.6-1.8 Gy, five fractions a week. The average total dose was 60.7 Gy. Complete response (CR)was obtained in 23 of the 38 patients (60.5 %)and partial response (PR)was obtained in the remaining 15 patients. The 3-year cause-specific survival rate was 39.6 % (overall, 29.4 %)(Fig.28). Three-year survival rates of CR and PR patients were 74 % and 0.0 %, respectively, and 2-year survival rate of PR patients
7.8 %; significant difference ($p<0.001$). The 3-year survival rates of the T1-3 and T4 patients (UICC,1987)were 73.0 % and 14.0 %, respectively; significant difference ($p<0.001$). Three-year survival rates of the 9 patients with tumors less than 5 cm in length, and of the 18 patients with tumors 5 to 10 cm were 80 % and 54.2 %, respectively. In the 11 patients with tumors more than 10 cm in length, 2-year survival rate was 9.0 %. The 3-year survival rate of the 18 patients with tumors less than 7 cm was 92.3 %, and 2-year survival rate of the 20 patients with tumors over 7 cm was 16.7
%; statistically significant difference ($p<0.001$). All 38 patients could be discharged in good condition and were able to take food orally.
This combination therapy could contribute not only to the survival rate, but also to the patients' quality of life.
Fig.28. The 3-year cause-specific and overall survival rates.

73. Decreased prefrontal dopamine D1 receptor binding in schizophrenia revealed by PET

Yoshiro Okubo*†, Tetsuya Suhara†, Kazutoshi Suzuki†, Kaoru Kobayashi†, Osamu Inoue†, Omi Terasaki*†, Yasuhiro Someya*†,Tsuyoshi Sassa*†, Tasuhiko Sudo†, Eisuke Matsushima*, Masaomi Iyo†, Yukio Tateno† & Michio Toru*

*** Department of Neuropsychiatry, Tokyo medical and Dental University School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan.**

† Division of Advanced Technology for Medical Imaging, National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba 263, Japan

The dopamine hypothesis for schizophrenia is strongly supposed by the antipsychotic effect of D2 dopamine receptor(D2R) antagonists. However, recent positron emission topography (PET) studies cast doubt bon the hypothesis that schizophrenis is related to increased striatal D2 densities. The D1 dopamine receptor (D1R), the most abundant dopamine receptor in the cortex, has been implicated in the pathophysiology of schizophrenia from the two view points. First, the reduced effect of D1R activity on D2R has been proposed in schizophrenia. Second, working memory dysfunction is assimed to be a fundamental cognitive disturbance in schizophrenia, and an important role of prefrontal D1R in working memory has been demonstrated. Using PET, we measured D1R and D2R in schizophrenics, and controls. Although striatal D1R and D2R, and the ratio of striatal D1R to D2R did not differ, D1R binding decreased in the prefrontal cortex (PFC) of drug-naive and drug-free patients. Reduction of prefrontal D1R was related to negative symptoms and poor performance in the Wisconsin Card Sort Test (WCST).
Thus, dysfunction in the D1R system i PFC may contribute to negative symptoms and cognitive deficits in schizophrenia.

74. Development of New Method for the Discriminative Measurement of Neuron and Glia Cell in Intact Brain with PET

Osamu Inoue1, Kaoru Kobayashi1, Takayuki Nakano1, Koichi Ando, Sachiko Koike, Yoshiya Furusawa, Junichi Ishida2 and Antony Gee3

(1 Osaka Univ., 2 Fukuoka Univ., 3 Arhus Univ. Hospital, Denmark)

Keywords: glia, cell, PET

It is becoming more important to measure glia cell function in an intact brain with PET for both diagnostics and basic brain research. The aim of this research is to find new types of radiotracers which allow measurement of the glia cell function in relation to the neutral function. We selected 13N-laveled ammonia as a glia cell mapping tracer, because 13N-ammonia has been reported to be taken up into the brain by a metabolic trapping mechanism. The converting enzyme which metabolizes ammonia to 13N-laveled amino- acid, glutamine synthetase, exists in glia cell and the metabolic-trapping rate seems to be rate limiting for the brain uptake of 13N-ammonia. In animal experiments using mice, modifications of dopaminergic, noradrenergic, serotonergic of GABAergic systems by various kinds od drugs significantly altered the brain uptake of 13N-amonia. These results indicated neurotransmission systems regulate the metabolic rates of ammonia to amino acid in glia cells. The combined use of 13N-ammonia and 15O-H2O would have some potency to reveal glia function in an intact brain. Another approach to develop new radiotracers is combined use of two different basic amines with different pKa values. We synthesized 11C-methamphetamine and b,b-difluoromethamphetamine and evaluated their brain kinetics using a pig. The results strongly indicated the importance of ionic interactions of amines with brain components rather than hydrophobic interactions. Long term retention of radioactivity of 11C-methamphetamine in pig brain was observed, whereas rapid entrance and clearance of 11C-b,b-difluoromethamphetamine was seen. The combined use of 11C-MAMP and 11C-b,b-difluoroMAMP is also of interest for new function brain mapping.

7 5．Imaging of Irradiation Effect on Neurotransmission System in Brain (Neuro-Receptor Mapping)

Tsunehiko Nishimura1, Osamu Inoue2, Kaoru Kobayashi2, Nobuhiko Takai3, Takayuki Nakano1, Tomoya Okada1, Yoshiya Furusawa, Mizuho Saito, and Koichi Ando

1. Biomedical Research Center, Osaka Univ. Medical School, 2. School od Allied Health Sciences, Faculty of Medicine, Osaka Univ., 3. Faculty of Pharmaceutical Sciences, Fukuoka Univ.

Key words: neuro-receptor mapping, irradiation, QNB, iomazenil, in vivo

The effects of proton irradiation on neuro-receptor binding in intact rat brain were investigated. ［3H］QNB, ［3H］SCH23390, ［125I］RTI-55 and ［125I］iomazenil were used as a selective radioligand for muscarinic acetylcholine receptors, dopamine D1 receptors, dopamine transporters and central benzodiazepine receptors, respectively.

Rats were anesthetized with pentobarbital and focal irradiation was performed on the right brain with 70 MeV proton beam (9.8 mm). One day after the irradiation, in vivo binding of ［3H］QNB, ［3H］SCH23390, ［125I］RTI-55 and ［125I］iomazenil was measured with autoradiography by using a Bio-Imaging analyzer(BAS3000).

All the radioligands studied had a significant increase in receptor binding in the irradiated side of the brain. On the other hand, *in vitro* binding of ［3H］QNB measured by using brain slices was unaltered by proton irradiation. In both cerebral cortex and striatum, apparent positive cooperatively of binding was seen, whereas a decrease in ［3H］QNB binding by competitive inhibition was observed in cerebellum and heart. Apomorphine-induced rotation was also observed in irradiated rats, which indicated neuro-transmission in the brain might be altered by proton irradiation. *In vivo* receptor mapping with PET or SPECT would have some potential to detect changes in brain function caused by radiation.

5. ENVIRONMENTAL SCIENCE

7 6． Potassium and Other Body Constituents of Japanese Male Adults

Masafumi Uchiyama,. Tomio Ishihara* , Y. Nakamura, N. Ogyu, S. Kobayashi and Takeshi A Iinuma
(* Gumma Technics College for Medicine)

Key words: Japanese, potassium, fat, whole-body, 40K internal dose, lean body mass

In the period from November 1963 to December 1994 the amount of potassium and 40K was measured for 3,459 Japanese in total using whole-body counters at the National Institute of Radiological Sciences. Statistic analysis for potassium and other body cnstituents was limited to data froma total od 261 male reseachers who were under similar working conditions and regularly measured for these parameters. In order to verify the results for the first group, measurements were also made for the same parameters in another adult male group (the second group) which was composed of 132 subjects from the prefectures where nuclear power plants were in operation or prefectures adjoining nuclear power plants. For each subject, height and body mass were measured every time. The whole body quantities of potassium and 40K were estimated by calibrating the measurement of the subject against that for a phantom which contained a known amount of potassium and 40K. Assuming that body constituents can be divided into fat and lean body mass in which potassium is contained, the amount of fat was estimated to be the difference between the body mass and the lean body mass. Based on the MIRD method, internal dose to the total body from 40K was computed. The subjects were divided into their 20's, 30's, 40's, and 50's and over. The measurements for each individual that were taken over one year were averaged for use in the following analysis. The average parameters were computed for 261 subjects in total for all ages in the first group. Results for two consecutive years were combined to accumulate a sufficient number of results. The following 6 periods were chosen in order to analyze secular changes in these parameters, years '63 and '64, '70 and '71, '75 and '76, '80 and
'81, '85 and '86 and '89 and '90.
Fig.29 indicates the temporal changes for the period from 1963 to 1964. Fat increased greatly during the observation period. Fat increased with age in the first group. Thus, this temporal increase seems to be artificial in part. The variance among individual subjects was about 50 %. The average weight of fat was 9.3 kg for the years 1963 to 1964. Body mass also showed a slightly increasing trend. The average was initially 56.4 kg. The change in individual subjects was about 12 %. By contrast, height, potassium content and lean body mass kept constant. Individual variances were 3.5 % for height and about 8 % for the other parameters. In the years 1963 and 1964, height , potassium and lean body mass were 164 cm, 126 g, and 47 kg, respectively. Dose due to 40k decreased during these observasion periods. Initially it was 194 μGya-1. The variance among individual subjects was 10%. For 1989 and 1990 the dose was 168 μGya-1 with a relative standard deviation of 10%. Average ages were 35 for 1963 and 1964 and 47 for 1989 and 1990. Within contemporaries, all parameters under study except fat decreased with age. The

regression coefficients for the first group were as follows: - 0.64 for height,
-0.02 for body mass, - 0.85 for potassium content, - 0.84 for lean body mass and - 0.67 for 40K internal dose. Fat indicated an increasing tendency with a regression coefficient of 0.54. The results from the second group supported these trends generally.

Figure caption

Fig. 29. Secular trends of whole-body potassium and other related parameters for Japanese male adults.

77. Nationwide Indoor Radon Survey in Japan

Kenzo Fujimoto, Sadayoshi Kobayashi, Masafumi Uchiyama, Masahiro Doi and Yuji Nakayama

Keywords: equivalent dose, indoor, nationwide, passive detector, radon, survey, wooden house

Final results of a nationwide indoor radon survey were obtained based on measurements at more than 7,000 houses throughout Japan. The measurements were conducted using passive radon detectors which were originally developed at Karlsruhe Nuclear Research Center in Germany. Each house was measured in two places namely a living room and a bed room for two successive periods of six months to obtain the annual average radon concentrations. The detector foils (polycarbonate) which were retrieved from the selected houses after each six month measurement, were subjected to a combined chamical-electrochemical etching process to develop etch pits due to alpha particles. The number of pits was then counted and converted to average radon concentrations during the measurements. The conversion factor was estimated by a series of calibration exercises which were made available by the Australian Radiation Laboratory, Melbourne, Australia and the Environmental Measurement Laboratory, New York, U.S.A. Annual average indoor radon concentrations in 5,717 houses were finally obtained as the output of the survey after scrutinizing raw data of more than 28,000 measurements. The histogram of radon concentrations in 5,717 houses is shown in Fig. 30. It shows roughly a log-normal distribution as commonly reported for radon concentration distribution. The arithmetic mean and its standard deviation of radon concentration are given as 20.8 and
18.8 Bq-m-3, respectively. The median, 90 percentile and 99.5 percentile are obtained as 16.0, 35.5 and 138 Bqm-3. Only 27 houses are over the action level (150 Bqm-3) set by the EPA in the U.S.A. These high radon concentration houses are distributed in the western part of Japan, except for one house found in Niigata Prefecture.

However, no radon prone area is found, although Hiroshima Prefecture has 6 high radon houses out of total 27 houses. All these high radon concentration houses are wooden houses except for two concrete houses in Okinawa Prefecture. Many higher radon concentrations are found in Chubu, Kinki, Chugoku and Shikoku districts, where winter is relatively mild and the air exchange rate of the houses in these regions is not low, the concentrations seems to have a relationship with the geology as found by the measurements of exposure rates due to terrestrial gamma rays. These areas are typical granite regions. The areas covered with volcanic ash, in Kanto District around Tokyo and Kagoshima Prefecture in Kyushu district show lower concentrations. The equivalent dose due to radon progeny in Japan is estimated to be 0.59 mSv y-1 applying the same assumptions given in UNSCEAR 1993 Report to the arithmetic mean obtained in this survey.

Publications:

1) Fujimoto, K., Kobayashi, S., Uchiyama, M., Doi, M., and Nakamura, Y.: Proceedings Vol. 2 of 1996 International Congress on Radiation Protection, Vienna, Austria, April 14- 19 (1996).
2) Fujimoto, K., Kobayashi, S., Uchiyama, M., Doi, M., and Nakamura, Y.: Hoken Butsuri, 32, 41-51, (1997).

7 8．Discrimination between the Uptake of Cs and K by Plant Roots

Yoshito Watanabe, Masae Yukawa and Yoshikazu Nishimura

Keywords: cesium, plant, potassium, discrimination factor

The transfer of 137Cs from soil to plants is affected by the characteristics of the soil, particularly the K level. Many short term studies have indicated that Cs and K are absorbed into root cells competitively via a common uptake system. In field studies, on the other hand, the effect of K in the soil on Cs uptake has been found to be complex and competitive inhibition for Cs uptake has not always been observed. This suggests that discrimination between Cs and K by the uptake system may change with soil conditions. The uptake of Cs and K was investigated to elucidate the change in discrimination between Cs and K. The first experiment was carried out over 2 weeks, the second over 2 hours.

In the 2 week experiment in a greenhouse, 10 day old pea, rice and Indian mastard seedlings were grown in tanks containing continuously aeraeted nutrient slutions. The solutions contained 5μM of Cs, and various concentrations of K ranging from 0.025 to 0.5 mM.After 2 weeks of cultivation, these plants were sampled for Cs and K concentrations by inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry. In the 2 hours experiment to evaluate Cs and K absorption,root segments were excised from the main roots of 10 day old maize seedlings grown in a solution culture containing 0.1 mM or 1 mM of K. The root segments were incubated for 2 hours in the aerated solution with Cs and K concentrations similar to the long term experiment as well as radioactive tracers 137Cs and 42K. The radioactivities in the root segments were measured with a NaI scintillation counter. The discrimination level between Cs and K was estimated using a discrimination factor (DF) which is defined as follows.

DF=([Cs]/[K]) influx into plant / ([Cs]/[K]) in culture solution

In the root segments incubated for 2 hours, although the Cs uptake decreased with the increase of the K level, the DF values were about 0.35 and were independent of the K level in the culture solution (Fig. 31). This suggests that Cs and K may be competitively absorbed, and that the discrimination in the uptake between Cs and K during the short term is relatively constant regardless of the K level. In the plants cultured for 2 weeks, on the other hand, the DF changed with differences in the K level in the culture solution.

With the increasing K levels, the DF tended to increase in the rice but decrease in the pea and the Indian mustard. In the 2 week experiment there were significant changes in the DF whereas in the 2 hour experiment there were not. This suggests that the discrimination between Cs and K may alter during an extended period of as long as 2 weeks. The long term change in the discrimination was supported by the comparison between the plants previously grown with different levels of K. The DF obtained from the 2 week experiment was significantly different between plants grown in a culture solution containing 0.1 mM of K and plants grown in a solution containing 1 mM of K. These results suggest that the discrimination in the uptake of Cs and K by plant roots may be affected by the level of K in the soil.

Fig. 31. Cs uptake and discrimination factor for the 2 hour experiment.

79. Ingestion and Body Ccontent of Elements of Importance in Radiological Protection - Sr Content in Bone

Hisao Kawamura, Yi Cao 1, Kunio Shiraishi, Toshiaki Ishii, Keiji Ozawa 2, Rusong Chen 1 and Masae Yukawa (1 China Institute for Radiation Protection, Taiyuan, Shanxi, China, 2 Nihon Axis, Hitachinaka)

Keywords: Sr, ingestion, bone, rib, whole skeleton, analysis, ICP-AES, general population

In view of scarcity of data on ingestion and body content of elements of importance in radiological protection, it is particularly needed to establish analytical methods for determining those elements in diet materials and human tissue samples in the Asian region. For instance, strontium (Sr) content in bone and diet, one of the bone volume seekers and of continuing interest, is yet to be studied by trace element analysis in many countries.

In the present work, determination of Sr in bone from China was carried out in relation to the IAEA-RCA Co-ordinated Research Programme on Reference Asian Man (Phase II). Rib samples were collected in Shanxi Province, ashed and pulverized at CIRP. Some of them were transferred to NIRS and aliquots of these bone ash samples, 0.2 g in weight, were subjected further to acid decomposition in a closed system. Digestion was repeatedly carried out using high purity nitric acid and a small quantity of hydrogen fluoride in a polytetrafluoroethyelene (PTF) pressure vessel heated in a commercially available microwave oven to obtain a clear-looking and precipitate-free sample solution. The resultant sample solutions were diluted appropriately and their Sr concentrations were determined by use of a multielement ICP emission spectrometer employing the analytical line at 407.8 nm. For quality control, NIST SRM 1400 Bone Ash was analyzed and excellent agreement with the certified value was confirmed. A sub- clean room and clean-air hood of Class 100 were used.

The Sr concentration of the rib samples was found to be ca. 230μg per g of ash in average. The average Sr-to-Ca concentration ratio for these rib samples was evaluated as ca. 0.61. There seems to be no data for direct comparison. However, this value for the rib samples from China, the number of which is still small (n=12), might be roughly compared with 0.51, the average Sr-to- Ca concentration ratio reported for the whole skeleton of Japanese. Further work is needed to obtain a representative value for the bone Sr content in China. Quantitative information may be necessary also for ingestion intake of Sr through diet since transfer of this alkaline earth element from food to bone is still of importance in biokinetics and internal dosimetry.

8 0．Internal Dose from Ingestion for Japanese adult

Kunio Shiraishi and Masayoshi Yamamoto* (*Kanazawa University)

Key words: internal dose, ingestion, intake, Japanese, radionuclide

The annual effective dose for Japanese adults was recently estimated to be 3.75 mSv (NSRA 1992) from all kinds of radiation exposures. In this effective dose estimation, estimates of radiation exposures to Japanese individuals were mostly used, but some values referred to UNSCEAR data (UNSCEAR 1988) involving Western individuals, due to the lack of body burden data for some nuclides in the Japanese population. Medical radiation exposure (2.25 mSv y-1) and exposure from natural sources of radiation (1.48 mSv y-1) were the major contributors for the Japanese populations. The sum of these two exposures represented 99.6% of the total annual effective dose. Exposure from natural sources consisted of external exposures (0.67 mSv y-1) and internal exposures (0.81 mSv y-1). Furthermore, approximately half (0.40 mSv y-1) of the latter was from radon and its decay products.

In this report, internal doses received only through ingestion of radionuclides for Japanese males are described. An attempt was made to estimate annual effective doses by using ingestion dose coefficients (dose equivalent per intake of unit activity, Sv Bq-1) ; internal doses through inhalation were not evaluated. First, the representative intakes of radionuclides for Japanese males were estimated from the literature. Second, the annual effective dose was calculated with the intake of individual radionuclides and ingestion dose coefficients (Sv Bq-1) reported by the International Commission on Radiological Protection (ICRP). The equation for calculating the annual effective dose per person is :

Annual effective dose = $\sum Ii \times 365 \times Di$ (1)

where Ii is the daily intakes of radionuclide i(Bq d-1), and Di is the ingestion dose coefficient (Sv Bq-1). Annual effective dose are summarized with fractional transfers to blood (f1) and ingestion dose coefficients (Sv Bq-1) in Table 1. Two annual effective doses were calculated, using the ingestion dose coefficients of ICRP 30 and ICRP 68 (ICRP 1978, 1994). Total annual effective doses from natural sources were found to be 0.42 and 0.32 mSv, respectively.

The differences between the two cases resulted from the ingestion dose coefficients of ICRP 30 and ICRP 68 for 210Pb and 210Po. Potassium-40, 210Pb, and 210Po were significant contributors to the totals. Contributions of each radionuclide to the total effective dose are shown in Table 1. Potassium-40 (63%), 210Pb (16%), and 210Po (16%) were the three biggest contributors, if the ingestion dose coefficients from ICRP 68 were used. Carbon-14 (4%) and 226Ra (0.8%) ranked fourth and fifth. The effective doses from the man-made nuclide 90Sr, 137Cs, 239,240Pu, and 241Am were negligibly small contributors. Although estimating effective doses through use of estimate of radionuclides is considered to have a low accuracy compared with use of body contents of radionuclides (UNSCEAR 1993), the total effective doses (0.42 mSv and 0.32 mSv) estimated in this study using the dose coefficients from ICRP 30 and ICRP 68, compared well with the value of 0.41 mSv obtained from use of body contents measurements (NSRA 1992). In particular, determination of radionuclides in the diet and food samples following

estimation of internal exposures is easier, more rapid, and more useful in an emergency situation than procedures using analysis of human tissues and organs.

Publications:

International Commission on Radiological Protection: Limits for Intakes of Radionuclides by Workers. ICRP Publication 30, Pergamon Press ,Oxford, 1978.

Nuclear Safety Research Association: Radiation in Life Environment. Nuclear Safety Research Association, Tokyo, 1992.

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR): Sources and Effects of Ionizing Radiation, 33-89, United Nations Publication, E94.IX.2, New York, 1993.

International Commission on Radiological Protection: Dose Coefficients for Intake of Radionuclides by Workers: Replacement of ICRP Publication 61. ICRP Publication 68, Pergamon Press, Oxford, 1994.

Shiraishi, K. and Yamamoto, M.: Health Physics., 71, 700-704, 1996.

CAPTION

Table 1. Dietary intakes of radionuclides and estimation of internal doses for Japanese males.

Nuclide	**Intake Bq d-1 per person**	**Ingestion dose coefficient, Sv Bq-1**		**Annual effective dose, mSv y-1**			
		ICPR30(1978)	**ICRP68(1994)**	**ICRO30**	**% of total**	**ICRP68**	**% of total**
3H	4.0	1.7x10-11(1)a	1.8x10-11(1)a	0.0005	0.0060	0.000026	0.0082
14C	56	5.6x10-10(1)	5.8x10-10(1)	0.011	2.8	0.012	3.7
40K	-	-	-	0.20b	48	0.20b	63
87Rb	2.3	1.3x10-9(1)	1.5x10-9(1)	0.0011	0.26	0.0013	0.39
210Pb	0.20	1.4x10-6(0.2)	6.8x10-7(0.2)	0.10	25	0.050	16
210Po	0.60	4.4x10-7(0.1)	2.4x10-7(0.1)	0.096	23	0.053	16
226Ra	0.025	3.1x10-7(0.2)	2.8x10-7(0.2)	0.0028	0.68	0.0026	0.80
230Th	0.0017	1.5x10-7(0.0002)	2.1x10-7(0.0005)	0.000093	0.022	0.00013	0.041
232Th	0.0017	7.4x10-7(0.0002)	2.2x10-7(0.0005)	0.00046	0.11	0.00014	0.043
234U	0.0099	7.1x10-8(0.05)	4.9x10-8(0.02)	0.00026	0.062	0.00018	0.055
235U	0.00041	6.8x10-8(0.05)	4.6x10-8(0.02)	0.000010	0.0024	0.0000069	0.0022
238U	0.0088			0.00020	0.049	0.00014	0.044

		6.3x10-8(0.05)	4.4x10-8(0.02)				
90Sr 137Cs 239,240Pu 241Am	0.066 0.064 0.0002 0.000037	3.6X10-8(0.3) 1.4X10-8(1) 1.2X10-7(0.0001) 5.9X10-780.0005)	2.8X10-8(0.3) 1.3X10-8(1) 2.5X10-7(0.0005) 2.0X10-7(0.0005)	0.00087 0.00033 0.000009 0.0000080	0.21 0.079 0.002 0.0019	0.00067 0.00030 0.00002 0.0000027	0.21 0.095 0.006 0.0009
Total effective dose				0.42	0.32		

a Figures in parentheses are fractional transfer to blood(f1).

b Estimated by whole body counters.

8 1． Determination of Lead in Red Wine by ICP-AES

M. V. R. Prasad* and Katsumi Kurotaki

(*Indira Gandhi Centre for Atomic Research, Kalpakkam, India)

Key words: lead content, wine, ICP-AES

Lead was used for ca. 40 years since 1939 as the anti-knocking agent of gasoline. Hence lead is an effective tracer for anthropogenic heavy metals and some radionuclides passing through the environmental cycle. Red wine which is produced in many places worldwide contains various unvolatile impurities due to the growing environment because it is produced from grapes without a distillation process. Then red wine is a good indicator to compare the environmental pollusion at various areas. It is important to investigate the lead content in wine produced at various places and years in order to understand the extent of the soil pollution.

250-500 cm3 of wine sample were evaporated, and then nitric acid was added and the residue was decomposed in a Teflon vessel in a stainless steel pressure bomb. Clear yellow solution was obtained and its quantity of Pb was determined by an inductively coupled plasma atomic emission spectrometer (ICP-AES). Table 7 shows the lead concentrations of wines produced in several countries and years. The Pb contents are 16, 38-86 and 163μg/dm3 for the wines produced in Japan, European areas and Chile,respectively. There was a decreasing tendency in Pb concentrations of wines produced in Europe during the last 5 years, and there was a very low level of radioactivity due to Cs- 137 in the Austrian wine of 1991. These suggested that the Pb pollution level of soil has decreased since use of leaded gasoline was stopped. Also radioactive pollution of soil could still be detected in neighbouring countries of the Ukraine 5 years after the Chernobyl accident. The Pb content of Japanese wine is very low. This seemed to be caused by the nature of the Japanese soil. It was likely that the very high Pb content of Chilean wine was mainly from Pb pollution of soil caused by copper mining and smelting.

Table 7. Concentrations of lead in wines produced in several countries.

Country (years)	Alcohol content /Volume%	Sample volume /cm3	Pb concentration /μg dm-3
Austria (1991)	12.0	250	86
Spain (1993)	12.5	500	38
Chile (1993)	12.5	500	163
Hungary(1994)	12.0	250	66
Austria (1995)	11.5	500	42
Japan (1995)	14.0	470	16

8 2 .Assessment of 137Cs Internal Exposure Dose to Japanese Infants due to the Chernobyl Accident

Hidenori Yonehara, Toshihiko Mitsuhashi* and Masafumi Uchiyama (*Tohoku National Agricultural Experiment Station)

Keywords: internal dose , Japanese, infant, Chernobyl accident, 137Cs, dose estimate

The results of epidemiological studies have pointed out that the radiation risk for youth might be higher than that for adults. Therefore it is important to assess the health risk to younger individuals precisely using appropriate methods. In order to assess the risk of the internal doses to Japanese infants from the Chernobyl accident, the doses from the accident and atmospheric nuclear weapon tests were estimated using a mathematical model and the results of the dose estimation were compared with each other. An average annual internal dose was calculated for three groups of infants. The first group was infants who were fed breast milk alone, the second was fed only artificial milk and third was fed both types. A standard growth rate for body mass and daily milk consumption were used to estimate the 137Cs internal exposure. 137Cs concentration of breast milk was estimated from 137Cs body burdens for male adults taking into account the difference in both the Cs intake amount and the biological half-time between male adults and nursing women. 137Cs concentration in artificial milk was derived from the results of the measurement by taking into account the market shares of various companies producing artificial milk. The body burdens of infants were estimated, using a single compartment metabolic model. Data were derived from the body burdens parameters for male adults measured by a whole-body counter. _ Considering the changes in the infant populations in_ each group due to changes in nutritional methods that occur with the growth_of infants, annual_ doses for the three groups were estimated in order to derive the average internal dose for all_the infant groups. The MIRD method was employed for the calculation of annual average internal doses. The results of the dose estimation are shown in Fig.32. In this figure, annual individual dose was given in μGy for breast-fed, bottle-fed and all the infants. The five year mean dose for all the infants for five year periods beginnig with 1968, 1973, 1981 and 1986 is also shown. The mean dose for the period 5 years after the accident was 0.30 μGy. Comparing it with the previous 5 years its increase was 40 %. The mean doses for the 2 five year-periods beginning with 1968 and 1973 were 1.4 and 0.87 μGy and these were estimated to be 3 and 5 times larger than the dose from the accident, respectively. Fr the periods studied with regard to the atmospheric nuclear weapon tests, bottle-fed infants suffered from heavier internal exposure due to 137Cs than breast-fed infants. There was no significant difference observed in the doses between breast-fed and bottle-fed infants for the period affected by the Chernobyl accident.

Fig. 32. Estimate of 137Cs internal dose to Japanese infants for the first 6 months from birth.

8 3．A Calibration Method for Whole-body Counters, Using Monte Carlo Simulation

Tetsuo Ishikawa, Masaki Matsumoto and Masafumi Uchiyama

Keywords: whole-body counting, 137Cs, counting Efficiency, Monte Carlo simulation, calibration method

The whole-body counting efficiency for a subject is generally dependent on the size of the subject. In order to estimate the body burden of a subject accurately, a counting efficiency appropriate to the body size of the subject should be used. It is not however practicable to prepare physical phantoms corresponding in size to every subject. By simulating the physial histories of photons, beginning with photon emission, the interaction of the photons with substances in the phantoms and ending with the pulse record in the detection system, the counting efficiencies for various body sizes can be estimated without physical phantoms. Also, by changing the detector parameters and geometry, the simulation can be applied to other apparatus.In the present study, a Monte Carlo simulation code was developed to estimate the counting efficiencies for 137Cs block phantoms and it was applied to a whole-body counter as NIRS. The code consists of mathematical models and parameters which are categorized into three groups: a geometrical model for phantoms and detectors; a photon transport model; and a detection system model. Photon histories were simulated with these models. The counting efficiencies for five 137Cs block phantoms of different sizes were calculated by the code and compared with those measured with a whole-body counter at NIRS. The phantoms corresponded to a newborn, a 5 month old, a 6 year old, an 11 year old and an adult. The differences between the measured and calculated values were within 6% (Table 8). For the adult phantom, the difference was 0.5%.The results suggest that the simulation code can be used to estimate the counting efficiencies for various body sizes. The calibration method developed in the present study is simpler and more flexible than the conventional method which needs several physical phantoms.

Publication:

Ishikawa, T., Matsumoto, M. and Uchiyama, M.: Radiat. Prot. Dosin., 64, 283-288, 1996.

Table 8 .Comparison between the measured and calculated and calculated couting efficiency for each phantom

Size of phantom	**Height (cm)**	**Weight (kg)**	**Activity (Bq)**	**Measured counting efficiency(10-3 cps/Bq)**	**Calculated counting efficiency (10-3 cps/Bq)**
Newborn	38.7	3.49	340	18.5	17.6
5 month old	65.7	10.8	500	12.7	12.1
6 year old	119	25.6	1000	8.41	7.95
11 year old	146	42.1	1500	6.71	6.37
Adult	162	66.2	1500	5.41	5.38

8 4. Influence of Radiation Exposure on Our Aociety and Epidemiological Study

Yasuhiro Yshimoto

Keywords: radiation ezposure, risk assessment, epidemiological study, nuclear power plant accident

A brief epidemiological review of the risk assessment of radiation was discussed with respect to two periods; before and after the establishment of the United Nations Scientific Committee on the Effects of Atomic Radiation. Selected topics were studies of the atomic bomb survivors and people living in the areas contaminated by the Chernobyl nuclear power plant accident. To ensure that the potential social benefits of epidemiology are maximized, both an ethical view as well as a scientific view were emphasized. It should be recognized that there are limitations with epidemiological studies that are based on the observations of man in which animal-experimental settings generally cannot be precisely controlled. Informing people about the current understandings of radiation exposure and the necessary cautions is needed to resolve social concern associated with low doses and low dose rates of radiation. Also guidelines for the investigation of clusters of adverse health events must be prepared. In the future a special strategy for decontamination might be needed for an unusual radiation exposure as a consequence of a nuclear power plant accident. Justification for such strategy of implementation can be determined only through the assessment of the effects both on the environment and on the health of humans after the accident.

8 5. An Epidemiological study of Japanese Radiological Technicians from 1969 to 1993

Shinji Yoshinaga, Takashi Aoyama* and Yasuhiko Yoshimoto (*Shiga University of Madical Science)

Keywords: occupational exposure, radiological technicians, Healthy Worker Effect, Standardized Mortality Ratio

The risk evaluation of the health effects due to radiation exposure is mainly based on epidemiological data from the atomic bomb survivors who had a single exposure to a high dose of radiation. For the effect of long-term exposure to low-dose radiation, specific scientific knowledge has not been obtained from epidemiological studies. Thus we carried out an epidemiological study on 12,133 male medical radiological technicians who received occupational exposure to low-dose radiation repeatedly over a long term. The vital status of the study population was confirmed by using the Japanese Family Register (Koseki) and death certificates with the special permission of the Regional Legal Affairs Bureau. 1,070 deaths from all causes were observed from 1969 to 1993. The numbers of deaths from cancer and leukemia were 437 and 20, respectively. When the age distribution of the study population was standardized by using mortality rates for all Japanese men, the SMR for all causes was 0.64 (95%CI=0.60-0.68) reflecting a strong Healthy Worker Effect. The SMR was 0.81(95%CI=0.74-0.89) for all cancers combined, and 1.31(95%CI=0.80-2.02) for leukemia.

When we calculated the SMRs by making all employees or technical occupation employees to be the standard population, the SMRs for all cancers combined and for many site-specific cancers increased. The SMR for leukemia almost reached a statistically significant level. Though this study result suggests that chronic exposure to low-dose radiation might increase the risk of leukemia mortality, a more detailed examination is necessary to explain the study results. This is because leukemia is a rare disease and some sub-types of leukemia are thought to be independent of radiation exposure. The average age of the survivors in the study population was 55.2-years at the end of the follow up period. Further follow-up is necessary as more important findings are expected to be obtained as the study population grows old.

8 6. The Influence of Knowledge on Risk Perception

Reiko Kanda, Yasuhiko Yoshimoto, Kenzo Fujimoto and Sadayoshi Kobayashi

Keywords: risk perception, risk ranking, radiation knowledge, risk communication, Japanese public

Research on risk perception has often been concerned with reaction of the public to modern technology. A major concern is the public fear of such new technology as nuclear power and genetic engineering. When the perceived risk was examined using a risk ranking technique, Japanese school teachers, university students and also NIRS staffs (female clerical staffs and researchers) viewed nuclear power to be much riskier than the objective estimation. Many technical experts have believed that this great fear results from an overestimation of risk by the public due to lack of scientific knowledge. So far, several studies reported the results to examine the correlation of the perception of some risk sources with knowledge about them, although their conclusions are inconsistent.

When the perceived risk by trainees on a radiation protection course in NIRS was examined, nuclear power was rated as the second and 14th among 30 risk items by those who majored in life sciences in college and by those in physics, chemistry or technology, respectively (Table 9). The perceived risk of nuclear power did not change among trainees by training offered fundamental knowledge about radiation during the course. On the other hand, the orders of smoking and alcoholic beverages rose considerably. Our results are consistent with the previous reports, i.e., what people learn initially about risk source has more important role in risk perception than what they learn later, and the increase of perceived risk is more easier than its decrease. Knowledge is now recognized as one of the factors which influence on risk perception. However, a special emphasis is now placed on risk communication in order to make partnership between communicators and receivers in the risk management.

Publication:

Kanda, R., Fujimoto, K. and Kobayashi, S. : Japanese Journal of Risk Analysis, 6,88-95, 1994.

Kanda, R., Joshima, H. and Kobayashi, S.: Japanese Journal of Risk Analysis , 7, 67- 73, 1995.

Kanda, R., Kobayashi, S. and Kanda, J.: Japanese Journal of Risk Analysis , (in press).

Table 9: Risk ranking before receiving radiation protection course training.

Risk ranking	Majors in life sciences	Majors in physics, Chemistry or technology
1(Highest)	Handguns	Handguns
2	Nuclear power	Private aviation
3	Private aviation	Hunting
4	Motorcycles	Motor vehicles
5	Motor vehicles	Motorcycles
6	Fire fighting	Police work
7	Large construction	Surgery
8	Hunting	Antibiotics
9	Police work	Large construction
10	Surgery	Commercial aviation
11	Smoking	Pesticides

14	X rays	Nuclear power
15	Mountain climbing	Food coloring
16	Antibiotics	Fire fighting
17	Food preservatives	Food preservatives
18	Contraceptives	Mountain climbing
19	Food coloring	Bicycles
20	Vaccinations	X rays
21	Power mowers	Power mowers
22	Alcoholic beverages	Alcoholic beverages
23	Skiing	Spray cans
24	Railroads	Railroads
25	Electric power	Electric power
26	Football	Vaccinatins
27	Bicycles	Skiing
28	Spray cans	Swimming
29	Swimming	Football
30(Lowest)	Home appliances	Home appliances

*The order of perceived risk is based on the geometric mean risk ratings within each activity or technology

87. Application of an Aquatic Microcosm as a Model Ecosystem for Assessment of Acidification Effect on Natural Ecosystem

Kiriko Tanaka-Miyamoto, Hiroshi Takeda, Shoichi Fuma, Kei Yanagisawa, Yoshikazu Inoue, Naoe Sato, Mayumi Hirano and Zen-ichiro Kawabata* (*Ehime University)

Key words: acidification effect, aquatic microcosm, ecological assessment, Escherichia coli, Euglena gracilis, model ecosystem, Tetrahymena thermophila

It is necessary to establish a reasonable method to assess the acidification effect on ecosystem that consists of various biological species and includes the interaction among them. It is not easy to assess any ecological effects from accumulated knowledge of the effect on single species. Experimental studies of model ecosystem might offer a way by which we can get valuable information that cannot be induced from either experiments for single species in a laboratory, or observation of complicated phenomenon in natural ecosystem. In this study an aquatic microcosm system was adopted as a model ecosystem for assessment of ecological effect.

The aquatic microcosm consists of three species of microorganisms in a small container like a test tube or a small plastic bottle. This system was composed by Kawabata et al. (1995), and the interaction among the three species has been investigated well. This microcosm consists of flagellate algae Euglena gracilis Z as a producer which has chloroprast for photosynthesis, ciliated protozoa Tetrahymena thermophila B as a consumer which grazes bacteria, and bacteria Escherichia coli DH5α as a decomposer which decomposes metabolite and dead bodies of the other two species. They can survive by exchanging materials with each other in the closed container with limited nutrients at the start of the microcosm system, and their population densities can be kept in a steady-state for a long time, usually for more than a year.

An experiment was carried out as follows: Each microorganism was preincubated following the method of Kawabata et al. Then three species of microorganisms were inoculated into a culture medium (0.05 % proteose peptone in a half strength of modified Taub and Dollar's solution) in test tubes. The culture medium was in advance acidified to pH 4.0 by adding a volume of equivalently mixed solution of nitric acid and sulfuric acid. The control medium was originally pH 7-8. Population densities of each organism were determined at various time intervals after starting incubation under a 2500 lx and 12-12 hrs. LD light regime at 25 ℃. The cell numbers of Euglena and Tetrahymena were counted microscopically, and those of E. coli were measured by counting colonies formed in the broth-agar medium.

Fig. 33 shows variation of the population density of E. coli and pH in the microcosm. In the control microcosm population density of E. coli increased and reached 106 cells/ml order on the sixth day after composition of the microcosm. However, population density of E. coli in the pH 4.0 microcosm did not increase and kept the initial order of 101 - 102 cells/ml till seven days passed. On the tenth day after the start of incubation, the population density of E. coli in the pH 4.0 microcosm increased to the same order of that of the control and pH reached 7.1. When only Euglena was cultured in the pH 4.0 medium it was observed that the pH was elevated by photosynthesis of Euglena. It is, therefore, concluded that in the pH 4.0 microcosm the pH was elevated by Euglena, and as a result, E. coli started growing. This is thought to

be an indirect effect which resulted from interspecific interaction that occurred in the model ecosystem. This microcosm system will be applied to comparatively evaluate the ecological impacts of other environmental toxicants hereafter.

Reference:

Kawabata, Z., Matsui, K., Okazaki, K., Nasu, M., Nakano, N. and Sugai, T. : J. Protozool. Res., 5, 23-26, 1995.it. Fig. 33. Variation of the population density of E. coli in the control and the pH 4.0 microcosm, and that of pH in the pH 4.0 microcosm.

8 8．Database on the Concentration Factors of Elements in Marine Organisms

Teruhisa Watabe and Setsuko Yokosuka

Keywords: concentration factor, biological concentration, database, marine organisms, cesium

The biological concentration of elements in marine organisms is one of the most important phenomena intervening in the transfer pathways to man of radioactivity released in the liquid effluents from nuclear facilities. In order to provide a comprehensive perspective on the biological concentration, the studies relevant to the issue carried out mainly in the Institute were reviewed. These studies included those carried out as an aquarium experiment with radioactive tracers, those obtained through radioactive survey programs, and those on the concentration analyses of stable elements in a variety of marine organisms. The extent of biological concentration of elements in aquatic organisms is usually expressed in terms of the 'concentration factor', which is widely used as a parameter in dose calculation exercises. The studies reviewed often provided explicitly the numerical values for the concentration factor or the data that could be converted into them. The values of the concentration factor were extracted, collated in a given format along with peripheral data such as bibliographic information etc., and saved in a database file on a personal computer so as to be easily sorted out according to the species of organisms or radionuclides. The database has been composed so far of approximately 2,600 data extracted from more than 40 reports and covering a total of 30 elements and a variety of species of organisms consumed as foodstuffs in Japan. The numbers of the data are not yet sufficient, however, to determine the parameter values for all the elements with full certainty, but large enough for a few elements such as cesium and so on. The numbers of the data recorded for cesium, for instance, amounted to 165, 56, 21 and 123, respectively for fishes, cephalopods, bivalves and brown algae. The frequency of the values of concentration factor showed nearly a normal distribution in the logarithmic scale for the respective species with the geometric mean values corresponding to 46, 89, 13 and 27 as shown in Fig. 34. These values were in good agreement with the reference values, namely 50, 10 and 10 for marine fishes, cephalopods and algae respectively, provided by the International Atomic Agency (IAEA) in its Safety Series No. 57. Further accumulation of data is being facilitated in order not only to make the existing parameter values more reliable, but also to provide the values additionally for the species of organisms which are served in local diets or as a dainty like sea squirts, sea urchins, sea cucumbers, etc.

Publication:

Radioactive Waste Management Center: Concentration factors of radionuclides in the marine organisms, Environmental parameter series 6, RWMC-96-P-18, Radioactive Waste Management Center, Tokyo, 1996.

8 9 .Separation and Concentration of Technetium using a Tc-selective Extraction Chromatographic Resin

Shigeo Uchida and Keiko Tagami

Key words: technetium-99, ruthenium, chromatographic resin, soil sample, ICP-MS

Technetium-99 is an artificial radionuclide with a long half-life of 2.1 x 105 y. Due to its high mobility in the terrestrial environment, it is one of the most important radionuclides for dose assessment. The main sources of environmental 99Tc are nuclear weapons tests and nuclear industries. The increasing use of 99mTc in nuclear medicine is introducing another important environmental source, since 99mTc directly decays into 99Tc with a
6.02 h half-life. Because 99Tc is a soft beta-emitting radionuclide and its concentration in the environment is very low, the chemical separation of Tc from environmental samples is necessary. We previously proposed a separation method for 99Tc in soil samples by inductively coupled plasma mass spectrometry (ICP-MS). The method includes a liquid- liquid extraction with cyclohexanone and CCl4 for separation of Tc and removal of Ru in an alkaline solution. Ruthenium should be removed because it has an isotopic abundance of 12.7% at mass of 99 which interferes with 99Tc measurement by ICP-MS. The extraction takes time and can not be performed automatically. Moreover, CCl4 will be banned for use because it acts as a destroyer of the ozone layer. Then we need a new separation method. Fundamental experimental data for using a TEVA·Spec resin instead of the liquid-liquid extraction were obtained for separation and concentration of 99Tc in solutions. In this study, 0.05M NaOH and 0.05M and 0.5M NaCl were used. One hundred mL each of the solutions containing 95mTc and 106Ru were introduced into the column, followed by 1 M and 12 M HNO3. The elution behaviour of Tc and Ru are shown in Fig.
35. Hardly any sorbed Tc was stripped from the TEVA·Spec resin column with 50 mL of 1 M HNO3 but it was easily desorbed with 5 mL of 12 M HNO3 for all solutions. In contrast, most of the Ru passed through the column for both NaCl solutions. However, for 0.05 M NaOH, Ru was sorbed on the resin and hardly any passed through with the solution. Most of the Ru sorbed on the resin was easily desorbed with 1 M HNO3 and some of it appeared in the eluate of 12 M HNO3. Ruthenium might exist as an anionic ion (RuO42-) in alkaline solution. The recovery of Tc for the three solutions were almost 100%. However, an alkaline solution was deemed not suitable for the separation of Tc using the resin; small amount of Ru appeared in the strip solution of 12 M HNO3.
The first 5 mL of the eluate with 12M HNO3 which contained 99Tc could be dried up on a hot plate under 80℃ without any loss of Tc. Then the residue could be dissolved in 5 mL of 2% HNO3. The concentration of 99Tc in this solution could be measured with ICP-MS.

Publication:

Uchida, S. and Tagami, K.: J. Radioanal. Nucl. Chem., Articles, 221, 35-39, 1997.

90. Analysis of Technetium-99 in Deposition and Soil Samples by Inductively Coupled Plasma Mass Spectrometry

Keiko Tagami and Shigeo Uchida

Key words: technetium-99, soil sample, rain and dry fallout, ICP-MS, cesium-137

Environmental amounts of 99Tc have been accumulating because of its long half-life of 2.1 x 105 y. This pure b-emitter (Emax = 294 keV) is produced in the fission of 235U or 239Pu with a relatively high fission yield of ca. 6%, and it is released to the environment through nuclear tests and nuclear industries. It is one of the most important radionuclides for dose assessment, because it has high mobility and low absorbability in the terrestrial environment. Rain and dry fallout samples were collected monthly using 5 polyethylene containers located on top of an institute building in Hitachinaka, Japan. Soil samples were collected from surface (0 - 15 cm) of agricultural fields, upland and paddy fields, in Japan. We focussed on the agricultural soils, because of high absorbability of Tc by plants. The samples were incinerated for 8 h at 450℃ to decompose organic matter. A brief explanation of the experimental method for each sample is given here. (1) Deposition samples were concentrated by heating on a hot plate without any loss of Tc. After adjusting to 1N nitric acid, the solution was heated 2 - 3 h at 70℃ on the hot plate. The solution was filtrated and then a liquid-liquid extraction with trioctylamine (TOA), which works as a liquid anion exchanger, was carried out. Tc was back-extracted from TOA/xylene into 1M potassium carbonate. (2) Technetium was separated from the incinerated soil sample by volatilization, i.e., by combustion of the sample and trapping of the element in a potassium carbonate solution. (3) The solution prepared in (1) or (2) was extracted into cyclohexanone to remove ruthenium which has an isotopic abundance of 12.7% at mass of 99. Then Tc in the organic layer is back-extracted into deionized water by addition of carbon tetrachloride. After the aqueous phase was adjusted to 2% nitric acid with super-analytical grade reagent (Tama chemicals, AA-100), 99Tc concentration in the solution was measured by ICP-MS (Yokogawa, PMS-2000). Recovery of Tc was determined with 95mTc (Du Pont, NaTcO4 in H2O). The recoveries of 95mTc in deposition and soil samples were 62 - 77% and 52 - 64%, respectively. The detection limit for ICP-MS in this method was 0.05 ppt (0.03 mBq/mL).

Technetium-99 in deposition of Hitachinaka city was from 0.4 to 0.9 mBq/m2/month. Although the detection limit for ICP-MS is low, the concentration of 99Tc in deposition sample in a month is difficult to measure. Table 10 shows the results of 99Tc and 137Cs concentrations of the soil samples. The soil samples were measured for their 137Cs activity by a Ge detector system (Seiko EG&G) before separation. The activity ratios of 99Tc /137Cs in the soil samples were calculated as 0.004 - 0.008. These values were higher than the theoretical one calculated from fission yield of 235U or 239Pu. The higher 99Tc /137Cs activity ratio in surface soil was influenced by that of deposition. However, it is still difficult to clarify the origin of 99Tc in the environmental samples. Further studies are needed to obtain the behaviour of Tc in the environment.

Publication:

Tagami, K. and Uchida, S.: Appl. Radiat. Isot., 47, 1057-1060, 1996.

Table 10. Concentrations of 99Tc in atmospheric and terrestrial samples

	Sample	Place	Year	n	99Tc activity	99Tc/137Cs(x10-2)	Reference
Atmospheric	Rain	U.S.A.	1961-1974	4	o.o6-1.3 mBq/L	0.11-2.5	Ehrhard K. C. and Attrep M. (1978)
sample	Rain	Spain	1984-1987	19	0.09-0.19 mBq/L (0.9-15.5 mBq/m2/rain)	0.3-12.3	Garcia-Leon M. et al. (1993)

	Rain	Monaco	1985-1986	3	0.02-0.09 mBq/L	0.23-1.9	Holm E. et al.(1988)
	Air	Spain	1965-1967	15	0.19-2.9 mBq/m3	0.4-1.0	Garcia-Leon M. et al. (1984)
	Air	Sweden	1981	8	0.48 mBq/m3	1.25	Holm E. et al. (1988)
	Deposition	Japan	1993-1994	7	<0.4-0.9 mBq/m2/month		This study
Terrestrial	Lichen	Sweden	1961-1975	12	20-190 mBq/m2		Holm E. et al.(1988)
sample	Soil	U.S.A.	1982	1	0.10 Bq/kg	0.053	Garland R. et al. (1983)
	Soil	Japan	1989	1	0.33 Bq/kg, dry		Morita S. et al. (1991)
	Soil	Japan	1991-1992	3	0.11-0.46 Bq/kg, dry	0.4-3.8	Tagami K. and Uchida S. (1993b)
	Soil	Japan	1993?	3	0.08-0.18 Bq/kg, dry	0.15-0.48	Morita et al. (1993)
	Soil	Japan	1991-1992	4	0.05-0.11 Bq/kg, dry	0.38-0.84	This study

9 1.Behavier of Radiocesium and Related Stable Elements in a Japanese Pine Forest

Satoshi Yoshida and Yasuyuki Muramatsu

Keywords: radiocesium, alkali elements, alkaline earth elements, mushroom, plant, forest

After the Chernobyl accident it turned out that forest ecosystem act as sink for radiocesium. In contrast to agricultural products, radiocesium contamination of forest products is very high, even ten years after the accident. Therefore, studies on the distribution and transfer of radiocesium in forest ecosystem are required. As chemical behavior of radiocesium is expected to be similar to that of stable Cs and the other alkali elements, analyses of stable elements must be useful to understand the long term behavior of radiocesium. However, the relationships among radiocesium and alkali elements in biological samples in forest ecosystems are not clear because of the lack of analytical data. In this study, analytical data of radiocesium and stable Na, K, Rb, Cs, Mg, Ca, Sr and Ba for mushrooms, plants and soils collected from a Japanese pine forest were summarized. Radiocesium was measured with a Ge-detector.Trace elements, Rb, Cs, Sr and Ba, were measured by inductively coupled plasma-mass spectrometry (ICP- MS). Major elements, Na, K, Mg and Ca, were analyzed by inductively coupled plasma- atomic emission spectrometry (ICP-AES).

Twenty-nine mushrooms and 8 plants collected from a Japanese pine forest on sandy soil with a thin organic layer were analyzed. In this forest, most of the radiocesium is derived from atmospheric nuclear weapons testing, particularly in the 1960s. In comparison with the elemental composition of plants, the mushroom composition could be characterized by the high 137Cs, Cs and Rb concentrations and low Ca and Sr concentrations. A good correlation ($r = 0.99$) between 137 Cs and stable Cs was observed for mushrooms (see Fig. 36). The 137Cs /Cs ratios were almost constant (137Cs/Cs = 134±36 Bq/mg in 1990). The ratios for plants and litter at the surface of the soil were almost the same as those for mushrooms. These findings suggest that 137Cs deposited to this pine forest due to nuclear weapons testing has been equilibrated with stable Cs biologically, and the available Cs fo rmushrooms and plants is recycling in the puine forest with a constant 137Cs/Cs ratio. In plant samples, good correlations were observed among K, Rb, Cs and Mg. Correlation between Ca and Sr was also good. These finding suggest the possibility of using the behavior of alkali and alkaline earth elements to predict the radiocesium and radiostrontium behavior in soil-plant systems in forests. In contrast, Cs was not correlated with K in mushrooms, indicating that the mechanism of Cs uptake differed from that for K. However, Rb showed a correlation with Cs ($r = 0.82$).

The analyses of stable elements have provided much information on the behavior of elements which are related to radionuclides. Such studies must be useful in predicting the migration of radionuclides in contaminated forests.

Publications:

Yoshida, SA. and Muramatsu, Y.: Intern. J. Environ. Anal. Chem., 1998(in press).

Yoshida, S. and Muramatsu, Y.: J. Environ. Radioactivity, 1998(in press).

6. APPENDIX

PUBLICATIONS

Division of Radiation Research Tomitani, T.: A Model of Optical Reflection on Rough Surface and its Application to Monte Carlo Simulation of Light Transport, IEEE Trans. Nucl. Sci.,NS-43(3), 1544-1548, 1996.

Hiraoka, T., Omata K., Fukumura A., Irifune T.* and Ootani, H.* Dosimetry for narrow beam-radiotherapy, Jpn. J. Radiol. Technol., VOl. 14, 66-72, 1996* (Tokyo Metropolitan College of Allied Medical Science)

Hirasawa M, Yamamoto M, Kawano K*, Yasuda N, Furukawa A : An experiment on extrasensory information transfer with electroencephalogram measurement(Part II). J Int Soc Life Info Sci, 14:185-195, 1996. (Nippon Med. Sch.)

Yamamoto M, Hirasawa M, Kawano K*, Kokubo H, Hirata T, Yasuda N, Furukawa A : An experiment on remote action against man in sense shielding condition
- Part2. J Int Soc Life Info Sci, 14:228- 248, 1996. (*Nippon Med. Sch).

Ueda, J., Ssaito, N.*, Shimazu, Y. and Ozawa, T.: A comparison of scavenging abilities of antioxidants against hydroxyl radicals, Arch. Biochem. Biophys., 333, 337-384, 1996. (*Kyoritsu College of Pharmacy)

Ueda, J., Saito, N.* and Ozawa, T.: ESR spin trapping studies on the reactions of hydroperoxides with Cu(II) complex, J. Inorg. Biochem., 64, 197-206, 1996. (*Kyoritsu College of Pharmacy)

Suzuki, K. T.*, Rui, M.*, Ueda, J., and Ozawa, T.: Production of hydroxyl radicals by copper- containing metallothioneine: Roles as prooxidant, Toxicol. Applied Pharmacol., 141, 231-237, 1996. (*Chiba Univ.)

Inano, H., Suzuki, K. and Wakabayashi, K*.: Chemoprevention of radiation-induced mammary tumors in rats by bezafibrate administered together with diethylstilbestrol as a promoter, Carcinogenesis, 17, 2641-2646,
1996. (*Gunma Univ.)

Division of Radiobiology and Biodosimetry

Kanda, R. and Hayata, I.: Comparison of the yields od translocations and dicentrucs measures conventional Giemsa staining and chromosome painting. Int. J. Radiat. Biol., 69, 701- 705, 1996.

Nihei, N., Ichikawa, T., Kawana, Y., Kuramochi, H., Kugoh, H., Oshimura, M., Hayata, I., Shimazaki, J., and Itoh, H.:

Genome Research Group

Takashi Imai, Masatake Yamauchi, Naohiko Seki, Takehiko Sugawara, Toshiyuki Saito, Yoichi Matsuda, Hiroki Ito, Takahiro Nagase, Nobuo Nomura and Tada-aki Hori: Genome Res. 6, 439-447, 1996.

Yoichi Matsuda, Takashi Imai, Tadahiro Shiomi, Toshiyuki Ssaito, Masatake Yamauchi, Toshiyuki Fukao, Yukihiro Akao, Naohiko Seki, Hiroko Ito,

Mapping of metastasis suppressor gene(s) for rat prostate cancer on the short arm of human chromosome 8 by irradiated microcell-mediated chromosome transfer. Genes, Chromosomes & Cancer 17, 260-268, 1996.

Kanai, T., Furusawa, Y., Fukutsu, K., Itsukaichi, H., Eguchi-Kasai, K., and Ohara, H.: Irradiation of mixed beam and design of spread-out Bragg peak for Heavy-ion radiotherapy. Radiation Res., 147: 78-85, 1997.

Ryoko Araki, Akira Fujimori, Kiyohiro Hamatani, Kazuei Mita, Toshiyuki Saito, Masahiko Mori, Ryutaro Fukumura, Mitsuoki Morimiyo, Masahiro Muto, Masahiro Itoh, Kouichi Tatsumi and Masumi Abe: Nonsense mutation at Tyr- 4046 in the DNA-dependent protein kinase catalytic subunit of severe combined immune deficiency mice. Proc. Natl. Acad. Sci. USA, 94 2438-2443, 1997.

B. Wang, K. Fujita, K. Watanabe, H. Mitani, T. Yamada and Shima: Induction of apoptosis cultured midbrain cells from embryonic mice. Radiation Res., 147; 304-308, 1997.

Division of Biology and Oncology Muto, M., Chen, Y., Kudo, E. and Mita, K..: Analysis of Early Initiating Event(s) in Radiation-Induced Thymic Lymphomagenesis Jpn. J. Cancer Res., 87, 247-257, 1996.

and Tada-aki Hori: Genomics, 34, 347-352, 1996.

Itoh, T., Shiomi, T., Shiomi, N., Harada, Y., Wakasugi, M., Matsunaga T., Nikaido, O., Friedberg, E.C. and Yamaizumi, M.: Rodent complementation group8 (ERCC8) corresponds to Cockayne syndrome complementation group A. Mutat. Res., 362, 167-174, 1996.

Miura, M., Nakamura, S., Sasaki, T., Takasaki, Y., Shiomi, T. and Yamaizumi, M.: Role of XPG and XPF/ERCC1 endonucleases in UV-induced immunostaining of PCNA in fibroblasts. Exptl. Cell Res., 226, 126-132, 1996.

Kasahara, M.*1, Hayashi, M.*1, Tanaka, K.*2, Inoko, H.*3, Sugaya, K., Ikemura, T.*4, and Ishibashi, T.*1: Chromosomal localization of the proteasome Z subunit gene reveals an ancient chromosomal duplication involving the major histocompatibility complex. Proc. Natl. Acad. Sci. ISA, 93, 9096-9101, 1996.

*1Hokkaido University School of Medicine, *2The University of Tokushima, *3Tokai University shool of Medicine, *4National Institute of Genetics

Miura, N., Ishida, N., Hoshino, M., Yamauchi, M., Hara, T., Ayusawa, D., and Kawakita, M.: Human UDP-galactose translocator: Molecular cloning of a complementary DNA that complements the genetic defect of a mutant cell line deficient in UDP-galactose translocator. J. Biochem., 120, 236-241, 1996.

Toyoguchi, T., Yamaguchi, K., Nakagawa, K., Fukazawa, T., Moriya, H. and Sakiyama, H. Change of complement C1s synthesis during development of hamster cartilage. Cell Tissue Res. 285, 199-204, 1996.

Kabaya, K.*, Obuchi, M*, Kuwaki, T.*, Shibuya, K.*, Watanabe, M.*, Nemoto, K., Yoshida, K. Kusaka, M.* Inoue, T. and Seki, M.*: Leukemia et. 20, 27-35, 1996. Kirin Brewery Co.Ltd.

Neoi, M., Mita, K., Ichimura, S., Cartwright, I. L.*1, Takahashi, E. *2, Yamauchi, M., Tsuji, H. Heterogeneous structure of the polyubiquitin gene UbC of HeLa S3 ccells. Gene, 175, 179-185, 1996. (*1Univ. of Cincinnati College of Medicime. *2Otsuka Pharmaceutical Co.,)

Hirobe, T.: Hydrocortisone is involved in regulating the proliferation and differentiation of mouse epidemical melanoblasts in serum-free culture in the presense of keratinocytes. Eur. J. Cell Biol., 71, 387-394, 1996.

Jwa Hidari, K. I. -P. *1, Ichikawa, S.*1, Fujita, T.*2, Sakiyama, H. and Hirabayashi, Y.: Complete removal of sphingolipids from the plasma membrane disrupts cell to substratum adjesopm of mouse melanoma cells. J. Biol. Chem., 271, 24, 14636-14641, 1996. (*1Riken, *2Setsunan Univ.)

Neoi, M., Cartwright, I. L., Mita, K., Ichimura, S.: Comparison of the 5' upstream region of the evolutionarily equivalent polyubiquitin gene of humans and Chinese hamsters. Gene, 179, 297-299, 1996.

Ptushkina, M,. Fierro-Monti, I., van den Heivel, J., Vasilescu, S., Birkenhager, R., Mita, K. and McCarthy, J.E.G.: Schizasaccharomyces pompe has a novel eukaryotic initiation factor 4F complex containing a cap-binding protein with the human elF4E C-terminal motif KSGST. J. Biochem., 271, 32818-32824, 1996.

Koike, M., Ishino, K., Kohno, Y., Tachikawa, T., Kartasova, T., Kuroki, T., Huh, N-H.: DMSO induces apoptosis in SV 40-transformed human keratinocytes, but not in normal keratinocytes. Cancer Letters, 108, 185-193, 1996.

Koike, M., Matsuda, Y., Miomori, T., Harada, Y- N., Shiomi, N., Shiomi, T.: Chromosomal localization of the mouse and rat DNA double- strand break repair genes Ku p70 and Ku p80/XRCC5 and their mRNA expression in various mouse tissues. Genomics., 38, 38-44, 1996.

Richards, R. I., Crawford, J., Narahara, K., Mangelsdorf, M., Friend, K., Staples, A., Denton, M., Easteal, S., Hori, T.-A., Kondo, I., Jenkins, D., Goldman, A., Panich, V., Ferakova, E. and Sutherland, G. R.: Dynamic mutation loci: allele distributions in different populations. Ann. Hum. Genet., 60: 391-400, 1996.

Miyagi, Y., Yasumitsu, H. Mizushima, H., Koshikawa, N., Matsuda, Y., Itoh, H., Hori, T., Aoki, I., Misugi, K. and Miyazaki, K.: Cloning of the cDNA encoding mouse "PP5/TFPI-2 and mapping of the gene to chromosome 6. DNA and Cell Biology, 15:947-954, 1996.

Mita, K., Morikyo, M., Ito, K., Sugaya, K., Ebihara, K., HOngo,E., Higashi, T., Hirayama, Y.and Nakamura, Y.: Comprehensive cloning of Schizosaccharomyces pompe genes encoding translation elongation fantors. Gene, 187, 259-266, 1997.

Kitagawa*1, M., Aizawa, S., Kamisaku, H., Sado, T., Ikeda, H.*2, and Hirosawa, K.*1: Distribution of Fv-4 resistent gene production in Friend leukemia virus resistant Fv-4r mouse strain. Exp. Hematol., 64, 1423-1431, 1996.
(*1Tokyo Medical Dental Univ., *2Natl. Inst. Animal Health)

Chen, Y., Kubo, E., Sado, T., Muto, M. Cytogenetic analysis of thymocytes during early stages after irradiation in mice with different suscep tibilities toradiation-induced lymphomagnesis. J. Radiat. Res., 37, 267-276. (1996)

Hamatani, K.*1, Matsuda, Y.*2, Araki., Itoh, H.*1 and Abe, M.: Cloning and Chromosomal Mapping of the Mouse DNA Dependent protein kinase Gene. Immunogenetics, 45:1-5, 1996
(*1Radiation Effects Research Foundation, *2Nagoya Univ.)

Furuno-fukushi, I., Tatsumi, K., Takahagi, M. and Tachibana, A.: Quantitative and quakutative effect of γ- ray dose-rate on mutagenesis in human
lymphoblastoid cells. Int. J. Radiat. Biol., 70, 209-217, 1996.

Zama, M.,: Discontinuous translation and structure of mRNA coding region. Nucleic Acids Symposium Series 35, 293-294, 1996.

Tachibana, A., Tatsumi, K., Masui, T. and Kato, T.: Large deletions at the HPRT locus associated with the mutator phenotype in a bloom's syndrome lymphoblastoid cell line. Molecular Carcinogenesis, 17, 41-47, 1996 Nakajima, T., and Yukawa, O.,: Radiation-induced translocation of
protein kinase C through membrane lipid

Sugaya, K., Sasanuma, S., Nohata, J., Kimura, T., Fukagawa, T., Nakamura, Y., Ando, A., Inoko, H., Ikemura, T. and Mita, K.: Gene organization of human NOTCH4 and (CTG)n polymorphism in this human couterpart gene of mouse proto-oncogene Int3. Gene, 189, 235-244, 1997.

Space and Particle Radiation Science Research Group S. Uehara, M. Miyoshi, K. Andou, S. Koike, K. Masuda: Early skin shrinkage, late skin contraction and leg contracture of mice exposed to two fractions of neutron. J. Jpn. Soc. Ther. Radiol. Oncol. 8: 21-27, 1996.
H. Namba, T. Irie, K. Fukushi, M. Iyo, T. Hashimoto amd K. Ando: Time course of changes in cerebral blood flow and blood-brain barrier integrity bu focal proton radiation in the rat. Neurological Research 18: 83-86, 1996.

MunakataN., MorohoshiF., HiedaK., SuzukiK., FurusawaY., ShimuraH., Ito, T. (1996) Experimental correspondence between spore dosimetry and spectral photometry of solar
ultraviolet radiation. Phorochemistry and Photobiology 63: 74-78.

Eguchi-Kasai, K., Murakami, M., Itsukaichi, H., Fukutsu, K., Kanai, T., Furusawa, Y., Sato, K., Ohara, H., and Yatagai, F.(1996) The role of DNA repair on cell killing by charged particles. Advances in Space Research 18(1/2): 109-118. Sato Y., Tanaka A., Furusawa Y., Matsumoto s., Murakami T., Soga F. (1996) Applications of 6 MeV/n heavy-ion beams to biological experiments. Reviews of Scientific Instruments 67: 2000-2004.

Takada, M. Nakamura, T*1. Baba, M.*2 Iwqasaki, T.*2 Kiyosumi, T.*2: Nucl. Instr. and Meth., A372, 253-261, 1996. *1Cyclotron

peroxidation in primary cultured rat hepatocytes. Int. J. Radiat. Res., 70, 473-480, 1996.

Araki, R., Fujimori, A., Hamatani, K., Mita, K., Saito, T., Mori, M., Fukumura, R., morimyo, M., Muto, M., Itoh, M, M., Tatsumi, K. and Abe, M.: Nonsense mutation at Try-4046 in the DNA-dependent protein kinase catalyticsubunit of severe combined imune deffeciency mice, Proc. Natl. Acad. Sci. USA., 94, 2428-2443, 1997.

Ishikawa, Y. and Aizawa, S., Ikeda, H. and Hirokawa, K.: Establishment of a therapeutic modell for retroviral infection using the genetic resistancemechanism of the host, Pathology. International., 46, 719-725, 1997.

Yoshida, K., Inoue, T., Nojima, K., Hirabayashi, Y. and Sado, T.: Calorierestriction reduces the incidence of myeloid leukemia induced by a singlewhole-body radiation in C3H/He mice, Proc. Natl. Acad. Sci. USA., 94, 2615-2619, 1997.

Wada, E., Sakiyama, H., Nakamura, M. and Kanegasaki, S.: Density Dependent Change of Myrustoylated Proteins in C3H10T1/2 Fibroblasts and Their Transformants, Cell Biochemistry And Function., 15, 19-26, 1997.

Division of Radiotoxicology and Protection

Takaki, H.*1, Fukuda, S., Iida, H., Sato, j.*1, Sato, R,*1 and Naito, Y.*1: Experimental studies on bovine hyena disease induced by administration of excessive vitamin AD3E premix, vitamin A, or vitamin D3. J. Vet. Med. Sci., 58, 311-316, 1996. (*1Iwate Univ.)

Radioisotope Center, Tohoku University *2Department of Nuclear Engineering, Tohoku University

M. Pinak, H. Yamaguchi and R. Osman: Molecular Dynamics (MD) Simulation of DNA Dodecamer with 5-hydroxy-6-cytosiny; Radical. Journal of Radiation Research vol. 371 No.1.

Yasuda, H., Ambe, S.*, Uchida, S.: Distribution coefficients of platinum group metals between soil solid and liquid phase, Environ. Technol., 17, 1151-1154, 996. (*Riken)

Takada, M. Shibata, T.*3 Uwamino, Y. :4 Nakamura, T.*2: Nucl. Instr. and Meth. A379, 293-306, 1996. *2Cyclotron Radioisotope Centre, *3Institute for Nuclear Study, University of Tokyo, TYanashi, Japan *4Institute of Physical and Chemical Research, Saitama, Japan

Baba, M.*1 Takada, T. Iwasaki, T.*1 Matsuyama, S.*1 Nakamura, T.*2 Ohguchi, H.*2 Nakao, N.*2 Sanami, T.*1 Hirakawa, N.*1: Nucl. Instr. and Meth., A376, 115-123, 1996. *1Department of Nuclear Engineering, Tohoku University, Sendai, Japan *2Cyclotron Radioisotope Centre, Tohoku University, Sendai, Japan

M. Iizuka, K. Ando, T. Aruga, H. Itsukaichi, K. Fukutsu, H. Nagasawa and H. Moriya: Effects of reoxygenation on repair of potentialy lethal radiation damage in cultured MG-63 osteosarcoma cells. Radiation Research, 147, 179-184, 1997.

Takaki, H.*1, Fukuda, S., Mori, R.*2, Kodaka, T.*2, Sato, R*1. and Naito, Y.*1, : Changes in bone metabolism and epiphysial griwth plate in bovine hyena disease induced by administration of vitamin AD3E premix or vitamin A. J. Vet. Med. Sci., 58, 407-412, 1996.
(*1Iwate Univ. *2Showa Univ.)

Yamada, Y., park, M. S.*, Okinaka, R. T.*, Chen, D. J.* : Molecular analysis and comparison of radiation-induced large deletions of the TPRT locus in primary human skin fibroblast. Radiat. Res., 145, 481-490, 1996. (*Los Alamos for National Laboratory)

Won, JH*, Fukuda, S., Sato, R.* and Naito, Y.*: Bone histomorphometric changes due to differences in calcium intake inder metabolic acidosis in rats. J. Vet. Med. Sci., 58, 611-616, 1996.
(*Iwate University)

Won, J., Oishi, N., Kawamura, T., Sugiwaka, T., Fukuda, S., Sato, R. and Naito, Y.: Mineral metabolism in plasma, ruine and bone of periparturient cows fed anionic diets with different calcium and phosphorus contents. J. Vet. Med.
Sci., 58, 1187-1192, 1996.

Delongchamp, R.R., Mabuchi, K., Yoshimoto, Y. Preston, D.L.: Radiat. Res., 147, 285-395, 1997.

Epidemiological Study Group of Nuclear Workers(Japan), First Analysis of Mortality of Nuclear Industory Workers in Japan, 1986-1992 J. HealthPhys., 32,
173-184, 1997.

K. Yoshida, T. Inoue, K. Nojima, Y. Hirabayashi, T. Sado: Calorie restoriction reduces the incidence of myeloid leukemia induced by a single whole-body radiation in C3H/He mice. Proc. Natl. Acad. Sci USA, 94, 2615-2619, 1997.

Quintern, L. E., Furusawa, Y., Fukutsu, K., Holtschmidt, H.: Charachterization and application of UV detector spore films: The sensitivity curve of a new detector system provides good similarity to the action spectrum for UV-induced erythema in human skin. J. Photochem. Photobiol. B: Biol. 37, 158-166,
1997.

Kanai, T., Furusawa, Y., Fukutsu, K., Itsukaichi, H., Eguchi-Kasai, K., Ohara, H.: Irradiation of mixed beam and design of spread-out Bragg peak for heavy-ion rafiotherapy. Radiat. Res.
147, 78-85, 1997.

Iizuka, M., Ando, K., Aruga, T., Furusawa, Y., Itsukaichi, H., Fukutsu, K., Nagasawa, H., Moriya, H.: Effects of reoxygenation on repair of potentially lethal radkation damage in cultured MG-63 osteosarcoma cells. Radiat. Res. 147, 179-184, 1997

Environmental and Toxicological Sciences Research Group

Yoshida, S., Muramatsu, Y., Tagami, K. and Uchida, S.: Dtermination of Major and trace elements in Japanese rock reference samples by ICP-MS. Intern. J. Environ. Anal. Chem., 63, 195-206, 1996.

Muramatsu, Y., Yoshida, SA., Uchida, S. and Hasebe, A: Iodine desorption from rice paddy soil. Water, Air and Soil Pollution., 86, 359-371, 1996.

Iimoto,T., R.Kurosawa,R.: A Pulse- Coincidence Rn-220 Monitor with Three Time-Gate. Environment International., 23, 1-6,7, 1996.

Ishikawa, T., Matsumoto, M. and Uchiyama, M.: A Calibration Method for Whole-body Counters, Using Monte Carlo Simulation. Radiat. Prot. Dosim., 64, 283-288, 1996.

Ishikawa, T., Mizushita, S. and Uchiyama, M.: Underestimation of 1311 Thyroid Burden doe to Systematic Bias in Measurements COnducted after the Chernobyl Accident. Radiat. Prot. Dosin., 67, 47-54, 1996.

Kanda, R. and Hayata, I.: Comparison of the Yields of Translocations and Dicentrics Using Conventional Giemsa Staining and Chromosome Painting. Int. J. Radiat. Biol., 69, 701-705, 1996.

Ma, J., Yonehara, H., Takashi Aoyama, Masahiro Doi, Sadayoshi Kobayashi, and Masanobu Sakanoue: Influence of air flow on the behabior of thoron and its progeny in a traditional Japanese house. Health Phys., 72(1): 86-91, 1997.

Nishizawa, K., Iwai,K., Matsumoto, T., Sakashita, K., Iinuma, T. A., Tateno, Y., Miyamoto, T. Shimura, A., and Takagi, H.: Estimation of The Exposure and a Risk-benefit Analysis for a CT System Desighned for a Lung Cancer Mass Screening Unit. Radiat. Prot. Dosim. 67, 101-108, 1996.

Sato, I., Matsusaka, N., Kobayashi, H., Suzuki, T., Sera, K., Futatsugawa, S., Nishimura, Y.: Effect of an Intraperitoneal Injection of Ca-EDAT and Zn-DTPA on Essential Trace Metal Concentrations in Rat Plasma, Int. J. PIXE, 5: 27-32, 1995.

Tagami, K. and Uchida, S.: Microbial role in immobikization of technetium in soil under waterlogged conditions. Chemosphere, 33, 217-225, 1996.

Tagami, K. and Uchida, S.: Agening effect on technetium in soil under aerobic and anaerobic conditions. Toxicol. Environ. Chem., 56, 235-247, 1996.

Yasuda, H., Ambe, S*, Uchida, s.: Distribution coefficients of platinum group metals between soil sollid and liquid phase. Environ. Technol., 17, 1151-1154, 1996. (*Riken)

Tagami, k. and Uchida, S.: Analysis of technetium-99 in soil and deposition samples by inductively coupled plasma mass spectrometry. Appl. Radiat. Isot., 47, 1057-1060, 1996.

A. Muramatsu, S. Uchida, M. Sumiya and Y. Ohmomo: Deposition velocity of gaseous organic iodine from the atmosphere to rice plants. Health Phys, 71, 757-762 (1996).

Schnetger* and Y. Muramatsu(*Visiting scientist from the Univ. Oldenburg, Germany): The analysis of halogens, with a special reference to I, in geological biological samples using pyrohydrolis from prparation and ICP-MS and IC for measurement. Analyst, 121, 1627- 1631 (1996)>

Ogiu, N., Nakamura, Y., Ijiri, I*1., Hiraiwa, K*2. and Ogiu, T.: A Statistical Analysis of the Internal Organ Weights of Normal Japanese People. Health Phys. 72(3) 368-383, 1997.

Sato, I., Matsusaka, N., Kobayashi, H., Tsuda, S. Nishimura, Y.: Radioisotopes, 45: 774-779, 1996.

Sato, I., Matsusaka, N., Kobayashi, H., Nishimura, Y.: Effects of Dietary Manganese Contents on Mn Metabolism in Mice. J. Rad. Res., 37, 125-132, 1996.

Shiraishi, K., Yamamoto, M.: Internal Dose from Ingestion for Japanese Adult Males. Health Phys., 71, 702-707, 1996.

Tokonami, S., Pan, J., Matsunoto, M., Furukawa, M., Fujimoto, K., Fujitaka, K. and Kurosawa, R.: Radon Measurements in Indoor Workplaces. Radiat. Prot. Dosin, 67, 143-146, 1996.

Tokonami, S., Iimoto, T., Ichiji, T., Fujitaka, K and Kurosawa, R.: Continuous Radon Monitor Using a Two- filter Method. Radiat. Prot. Dosim, 63, 123-126, 1996.

Tokonami, S., Iimoto, T., Ichiji, T., Tanaka, N., Kurosawa, R., : Integrated Measurement of Equilibrium Equivalent Radon and Thoron concentrations Using Cellelose Nitrate Film. Radiation Measurements., 26, 689-699, 5, 1995. Tokonami, S., Iimoto, T., Kurosawa, R. : Continuous Measurement of Equilibrium Factor and fp Value of Radon in the Environment. Environment International., 23, 1-6,6, 1996.

Tagami, K. and Uchida, S.: Concentration of Global Fallout 99Tc in Rice Paddy Soils Collected in Japan. Environ. Pollut. 95, 151- 154, 1997.

Ikeda, H., Tagami, K. and Fukuda, N.: A Study on a Simple Passive Hydroponic System for Melon Production, J. Japanese Society for Horticultural SAcience, 64, 839-844, 1996.

Low Dose Radiation Risk and Carcinogenesis Research Group Kobayashi, S., Otsu, H., Noda, Y., Ogiu, T.:

Comparison of dose-dependent enhancing effects of γ-ray irradiation on urethan-induced lung tumorigenesis in athymic nude (nu/nu) mice and euthymic (nu/+) littermates. J. Cancer Res. Clin Oncol., 122, 231-236, 1996. Shimada, Y., Ito.* : Heterogeneous ageing of macrophage-lineage cells in the capacity for TNF production and self renewal in C57BL/6 mice. Mech. Aging Develop., 87, 183-195, 1996. (*Tokyo Metropolitan Geriatric Hospital) Neoi, M., Mita, K., Ichimura, S., Cartwright, I.L.*1, Takahashi, E.*2, Yamaushi, M., Tsuji, H.: Heterogenous structure of the polyubiquitin gene UbC of HeLa S3 cells. Gene, 175, 179-185, 1996. (*1Univ. Cincinnati, USA, *2Otsuka Pharmaceut. Co.)

Ajiro, K,*1, Yasuda, H.*2, Tsuji, H.: Vanadate triggers the transition from chromosome condensation to decondensation in a mitotic mutant (tsTM13). Eur. J. Biochem., 241, 923- 930, 1996. (*1Aichi Cancer Cent. Res. Inst., *2Tokyo Col. Pharmacy)

Teraoka, H.*1, Yumoto, Y.*2, Watanabe, F., Tsukada, K.*1, Suwa, A.*2, Enari, M.*3, Nagata, S.*3: CPP32/Yama/apopain cleaves the catalytic component of DNA-dependent protein kinase in the holoenzyme. FEBS Letters, 393, 1-6, 1996. (*1Tokyo Med. Dent. Univ., *2Keio Univ. Med. School., *3Osaka Univ. Med. School)

Tokonami, S., Iimoto, T., Ichiji, T., R. Kurosawa, R.: Calculation Procedure of Potential Alpha Energy Concentratino with Continuous Air Sampling. Health Physics, 71, 5, 1-7, 1996.

Uchiyama, M., Nakamura, Y. and Kobayashi, S.: Analysis of Body-burden Measurements of 137Cs and 40K in a Japanese Group over a Period of 5 years Following the Chernobyl Accident.
Health Phys., 71, 320-325, 1996.

Yukawa, M., Kimura, A., Watanabe, Y., Sakurai, S.: Determination of elements in bean sporout determined by PIXE analysis Internal J. PIXE, 5: 69-77,
1995.

Low Dose Radiation Risk and Carcinogenesis Rersearch Group Kobayashi, S., Otsu, H., Noda, Y., Ogiu, T.: Comparison of dose-dependent enhancing effects of γ-ray irradiation on urethan-induced lung tumorigenesis in sthymic nude (nu/nu) mice and euthymic (nu/+) littermates. J. Cancer Res. Clin Oncol., 122, 231-236, 1996.

Shimada, Y., Ito, H.* : Heterogeneous ageing of macrophage-lineage cells in the capacity for TNF production and self renewal in C57BL/6 mice. Mech. Aging Develop., 87, 183-195, 1996. (*Tokyo Metropolitan Geriatric Hospital)

Nenoi, M., Mita, K., Ichimura, S., Cartwright, I.L.*1, Takahashi, E.*2,

Ogiu, N., Nakamura, Y., Ijiri, I.*1, Hiraiwa, K.*2, Ogiu, T.: A statistical analysis of the internal organ weights of normal Japanese people. Health ohys., 72, 368-383, 1997. (*1Kagawa Med. School, *2Fukushima Med. College)

Kobayashi, S., Nishiumura, M., Shimada, Y., Suzuki, F.*1, Matsuoka, A.*2, Sakamoto, H.*2, Hayashi, M.*2, Sofuni, T.*2, Sado, T., Ogiu, T.: Increased sensitivity of scid heterozygous mice to ionizing radiation. Int. J. Radiat. Biol. (in press) (*1Hiroshima Univ., *2Natl. Inst. Health Sci.)

Division of Radiation Medicine

Tsuji, H. Morita, S. Miyamoto, T. Mizoe, J. Mukai, M. Nakano, T. Kato, H. Kamada, T. Ishikawa, A. Matsuoka, Y.: Preliminary Results of Phase I/II Carbon-Ion Therapy at the National Institute of Radiological Sciences. Journal of Brachytherapy International, 13, 1-8, 1997.

Yoshioka, H. Onaya, H. Itai, Y. Nishimura, H. Matsumura, A. tsunoda, T. Kandatsu, S. Kaga,
M. Yoshikawa, K. Kato, H. and Tsujii, H.: Comparison Between Magnetization Transfer Contrast and fast spin-echo mr Imaging og digenerative disease of the cervical spine at 0.3t., Magnetic Resonance Imaging, 15, 1-8, 1997.

Division of Advanced Technology for Medical Imaging

Yamanouchi, M*, Yoshida, K., Niwayama, H.*, Nakagawa, K.*, Aioi, S.*, Shikama, N.*, Himi, T.*, Imazeki, K.*, Itoh, Y.*, and Masuda, Y.: Effect of the Diration of Fasting on Myocardial Fluorodeoxyglucose Positron Emission Tomography Images in Normal Males. Japanese Circulation Journal., 60(6), 319-327, 1996. (*Chiba Univ.)

Obata, T.*1, Ikehira, H.*2, Ueshima, Y.*3, Kato, H., Koga, M., and Yoshida, K.:

Yamauchi, M., Tsuji, H.: Heterogenous structure of the polyubiquitin gene UbC of HeLa S3 cells. Gene, 175, 179-185, 1996. (*1Univ. Cincinnati, USA,
*2Otsuka Pharmaceut. Co.)

Ajiro, K.*1, Yasuda, H. *2, Tsuji, H.: Vanadate triggers the transition from chromosome condensation to decondensation in a mitotic mutant (tsTM13). Eur. J. Biochem., 241, 923-930, 1996. (*1Aichi Cancer Cent. Res. Inst., *2Tokyo Col. Pharmacy)

Teraoka, H.*1, Yumoto, Y.*2 Watanabe, F., Tsukada, K.*1, Suwa, A.*2, Enari, M.*3, Nagata, S. *3:
CPP32/Yama/apopain cleaves the catalytic component of DNA-dependent protein kinase in the holoenzyme. FEBS Letters, 393, 1-6, 1996. (*1Tokyo Med. Dent. Univ., *2Keio Univ. Med. School.,*3Osaka Univ. Med. School)

Ogiu, N., Nakamura, Y., Ijiri, I.*1, Hiraiwa, K. *2, Ogiu, T.: A statistical analysis of the internal organ weights of normal Japanese people. Health Phys., 72, 368-383, 1997. (*1Kanagawa Med. School, *2Fukushima Med. College)

Kobayashi, S., Nishimura, M., Shimada, Y., Suzuki, F.*1, Matsuoka, A,*2, Sakamoto, H.*2, Hayashi, M.*2, Sofuni, T.*2, Sado, T., Ogiu, T.: Increased sensitivity of scid heterozygous mice to ionizing radiation. Int. J. Radiat. Biol. (in press) (*1Hiroshima Univ., *2Natl. Inst.Health Sci.)

Division od Radiation Health Ichikawa, S., Sakiyama, H., Suzuki, G., Hidari, K.I.J., and Hirabayashi, Y.: Expression cloning of a cDNA for human ceramide glucosyltransferase that catalyzes the first glycosylation step of

Noninvasive analysis of water movement in rat testis using deuterium magnetic resonance imaging. Magnetic Resonance Imaging., 14(1), 115-119, 1996. (*1Yokohama Muinicipal Univ.
*2Chiba Univ. *3Asahi Medical Co.)

Suhara, T., Farde L., Halldin C., Nagren K., Karlsson P.: Effext of cocaine on［11C］ norepinephrine and［11C］β-CIT uptake in primate peripheral organs measured by PET. Ann Nucl Med, 10: 85-88, 1996.

Nagren K., Halldin C., Swahn C-G., Suhara, T., Farde L.:［11C］metaraminol, a false neurotransmitter. Preparation, Metabolite Studies and Positron emission tomography examination in monkey. Nucl. Med. Biol., 23: 221-227, 1996.

Farde L., Suhara T., Halldin C., Nyback H., Nakashima, Y., Karlsson P., Ginovart N., Bymaster FP., Shannon HE., Suzdak PD., Sauerberg P. PET-study of M1-agonists［11C］xanomeline and［11C］butylthio-TZTP in monkey and man. Dementia, ': 187-198, 1996. Irie, T., Fukushi, K., Namba, H., Iyo, M., Tamagami, H., Nagatsuka, S. and Ikota, N.: Brain Acetylcholinesterase Activity: Validation of a PET Tracer in a Rat Model of Alzheimer's Disease.: J. Nucl. Med., 37, 649-655, 1996.

Namba, H., Irie, T., Fukushi, K., Iyo, M., Hashimoto, T. and Ando, K.: Time courses of changes in cerebral blood flow and blood-brain barrier integrity by focal proton radiation in the rat.: Neirological Res., 18, 83-86, 1996.

glycosphingolipid synthesis. Proc. Natl. Acad. Sci. USA. 93, 4638-4643, 1996.

Nomura, M., Nakata, Y., Inoue, T., Uzawa, A., Itamura, S., Nerome, K., Akashi, M., and Suzuki, G.: In vivo induction of cytotoxic T lymphocytes specific for a single epitope introduced into an unrelated molecule. J. Immunol. Methods. 193, 41-49, 1996.

Makoto, A., Int. J. Cancer, 68, 384-390, 1996.

Biological Research Group Ozawa, T., Miura, Y., and Ueda, J.: Oxidation of spin-traps by cholrine dioxide (CIO2) radical in aqucous solutions: First ESR evidence of formation of new nitroxide radicals. Free Radic. Biol. Med., 20, 837-841, 1996.

Ueda, J., Saito, N.,* and Ozawa, T.: Detection of free radicals produced from reactions of lipid hydroperoxide model compounds with Cu(II) complexes by ESR spectroscopy. Arch. Biochem. Biophys., 325, 65-76, 1996. (*Kyoritsu College of Pharmacy)

Inano, H., Suzuki, K., Onoda, M. and Yamanouchi, H.*.: Susceptibility of fetal, virgin, pregnant and lactating rats for the induction of mammary tumors by gamma rays. Radiat. Res. 145, 708-713, 1996. (*Wakayama Med. College)

Foged C., Halldin C., Hiltunen J., Braestrup C., Thomsen C., Hansen H. C., Suhara, T., Pauli S., Swahn C.-G., Karlsson P., Larsson S., Farde L.: Development of 123I-labelled NNC13-8241 as a radioligand for SPECT visualization of benzodiazepine receptor binding. Nucl. Med. Biol, 23: 201-209, 1996.

Muller L., Halldin C., Lundkvist C., Swahn C.-G., Foged C., Hall H., Karlsson P., Ginovart N., Nakashima Y., Suhara T., Farde L.: Development of radioligands for the dopamine transporter J. Radioanalytical and Nuclear Chemistry, 206: 133-144 1996.

Inoue T, Watanabe S, Masuda Y, Yoshida K, Imai H, Imazeki Y, Ishizuka Y, Koga M, Kato H, Ikehira H, Tateno Y.: Eveluation od blood flow patterns of true amd false lumens in dissecting aneurysms using MR phase-contrast techniques. Clinical Imaging 1996; 20: 262-268.

Yoshida K, Mullani N, Gould KL.: Coronary flow reserve by PET simplified for clinical applications using rubidium-82 or nitrogen-13 ammonia. J. Nucl. Med, 1996: 37: 1701-1712.

K. Nishizawa, K. Iwai*1, T. Matsumoto, K. Sakashita, T. A. Iinuma&, Y, Tateno, T. Miyamoto, A. Shimura*2 and H. Takahagi*3: Estimation of the Exposure and a Risk-Benefit Analysis for a CT System designed for a Lung Cancer Mass Screening Unit. Radiation Protection Dosimetry 67(2): 101-108, 1996.

National Institute of Radiological Sciences, *1Department of Radiology, School of Denstry Nihon University,&Saitama Institute of technology, *2Chiba Anti-Tuberculosis Association, *3Hitachi Medical Corporation

Ikota, N. and Hama-Inaba, H. Chiral Pyrrolidine Derivatives as Catalyst for the Enantioselective Addition of Diethylzinc to Aldehydes. Chem. Pham. Bull., 44, 587-589, 1996.

Fujiwara-Hirashima, C.*, Anzai., Takahashi, M., **, Kirino, Y.*: A voltage- dependent chloride channel from Tetrahymena ciliary membrane incorporated into planner lipid bilayers. Biochem. Biophys. Acta, 1280, 207-216, 1996. (*Kyushu Univ., **Univ of Tsukuba)

Ueda, J., Miyazaki, M.*, Matsushima, Y.*, and Hanaki, A.**: Glycylsarcosyl-L- histidylglycine, A peptide with a "breakpooint" in the complex formation: hydrolysis of the complex [CuH-1L], J. Inorg. Biochem., 63, 29-39, 1996. (*Kyoritsu College of Pharmacy, **Shizuoka Univ.)

Tsutsumi, M.*, Sadanaga, H.*, Lee, S.*, Sugihara, G.*, Anzai, K.: Studies on association behavior of transmembrane segment of influenza virus M2 protein in lipid bilayers. Peptide Chemistry, 1995, 225-228, 1996. (*Fukuoka Univ.)

Murayama, H., Nohara, N : Local nouse property in positron volume imaging and optical condition for the signal-to-nouse ratio of the 3D reconstructed image. Phys. Med. Biol., 42, 231-249, 1997.

Obata T*, Shishido F, Koga M, Ikehira H, Kimura F, and Yoshida K.(*Dependent of Physiology, Yokohama City University School of Medicine): Three-vessel study of cerebral blood flow using phase-contrast magnetic resonance imaging: Effect of physical characteristics. Magnetic Resonance Imaging 1996; 14(10): 1143-1148.

Laboratory for Radioecology Yamada, M., Aono, T. and Hirano, S.: 239+240Pu and 137Cs distributions in seawater from the Yamato Basin and the hydrolysis of the complex [CuH-1L], J. Tsushima Basin in the Japan Sea. J. Radioanal. Nucl. Chem., Articles, 210(1), 129-136, 1996.

Nozaki, Y., Zhang, J. and Hiroshi, H.: The tractionation between Y and Ho in the marine environment. Earth Planet. Sci. Lett., 148, 329- 340, 1997.

List of Keywords

3D verification system
8q24.1
12C
40K
137Cs
absorbed dose
absorbed dose comparison
acidification effect
active form specific antibody
alkaline earth elements
alpha wave amplitude
linac
linear qudratic
lipid
internal dose
lipid peroxidation
liposome
low-dose radiation
low dose rate
LPS
LSAB method
LSCT
lung cancer

analysis
anti-sense cDNA
APRT
aquatic microcosm
ascorbic acid
asymmetric addition
ataxia telangiectasia
ATM
atomic force microscope
B6C3F1 mice
BCT
beam deceleration
beam quality
biological concentration
biological effect
bone
bone histomorphometry
bone marrow cells
bone marrow chimeras
breast cancer cell line
bulk etch rate
calibration method
caloric restriction
CAR bacillus
carbon beam
carbon ion beam
cDNA catalogue
cell culture
cell cycle
cervical cancer
cesium
cesium-137
charged particle therapy
chemical dosimetry
Chernobyl accident
chiral ligand
chondrocytes
lung cancer cell line
lymphocyte
mammary cells
marine organisms
medaka
medical accelerator
medical exposure
medical record management
melanocyte
mice
microsomal membranes
model ecosystem
Monte-Carlo simulation
mouse
mouse model
MRI
mRNA structure
MTT assay
mushroom
mutagenesis
mutant brain
mutant chromosome instability
mutation induction
myeloid leukemia
nationwide
neon ion beam
neutral tube
neuro-receptor mapping
nitric oxide
nitroxyl radical
non-rejoining chromatin break
Northern analysis
NPAT
nuclear damage assay(NDA)
nuclear fragmentation reaction
nuclear medicine
nuclear power plant accident

chromatographic resin
chromosome11q-22-23
chromosome aberration
clinical trial
colony formation
combination therapy
complement C1s
concentration factor
concerted evolution
conformation therapy
copper(II) complexes
co-simulation
counter telescope
counting efficiency
CR-39
cross section
cryoperservation
CTLA-4
cumulative excess mortality
culture
CXCR4 expression
database
DDREF
delayed olating
deletion
development
diacylglycerol
diethyl zinc
differentiation
discrimination factor
DNA-strand scission
dose estimate
dose planning system
dose rate effect
dosimetry
numerical simulation
occupational exposure
ocular melanoma
ODC mRNA
OK-432
oxidative stress
partial dominance
particle identification
passive detector
PCC
penumbra
perfusion scan
PET
phosphatidylinositol kinase
photon
plant
polyamine
polyhydroxylated pyrrolidine
polyubiquitin gene
positioning
positron
positron emission tomography
potassium
premature death
proliferation
protein kinase C
proton beam therapy
pulmonary function
purine
putrescine
QNB
radiation
radiation carcinogenesis
radiation exposure
radiation-induced myeloid leukemia
distamycin A-inducible fragile site
pyrimidine
DNA-PKcs
qigong

dosimetry intercomparison radiation induced liver injury
D-ribonolacton radiation-induced lymphomagenesis
DVH radiation knowledge
ecological assessmentradical scavenging activity
EEG radioimmunoassay
embryo radiological technicians
epidemiological study radionuclide
equivalent dose radioprotection
Escherichia coli radiocesium
esophageal cancer radiosensitivity
etch pit radon
Euglena gracilis rain and dry fallout
extrasensory rat hepatocyte
fat RBE
fish reactive oxygen species
fission yeast remote action
flow cytometry relative risk
forest reproducibility
FRA8E respiration-gated irradiation
fractinated radiation respiratory motion
fragment respiratory movement
Fricke dosimeter retrotransposon
γ-rays RF-KO extraction
gated CT RFLP
gated irradiation rib
general population ribosomal protein gene
genetic variation ridge filter
genome analysis right frontal region
genomic DNA cloning risk assessment
genomic instability risk-benefit analysis
glia cell risk commnication
glutathione peroxidase risk perception
glutathione reductase risk ranking
granulocyte RNA polymerase II largest subunit group G xeroderma pigmentosum scid mouse
growth failure scid mutation
Healthy Worker Effect SDF-1

heavy charged particle
heavy charged particle therapy
heavy ion cancer treatment
heavy ion particle beam
heavy ion radiotherapy
heavy ion therapy
heavy ion treatment
hematopoietic stem cells
hematocellular carcinoma
HIMAC
HLE
HLF
HPRT
hydrocortisone
hydroxyl radical
hypoxic
ICP-AES
ICP-MS
IL-1
image fusion
image reconstruction
immobilization
immunohistochemistry
indoor
infant
information transfer
intake
interleukin-3 gene
internal dose
intracisternal A-particle
intron
in vivo
in vivo EPR
iomazenil
ionization chamber
ion selsctive electrode
sense shielding
serum
SOBP
soil sample
spallation
spermatozoa
spider silk fibroin
spontaneously hypertensive rat
Sr
SSAT mRNA
Standardozed Mortality Ratio
steroid
strain difference
strain maintenance
subcellular locarization
subconscious
superparamagnetic iron oxide
suggestion
survey
synchrotron radiation
synthesis
target duplication
target fragmentation
technetium-99
temporal pattern
Tetrahymena thermophila ingestion
theory
thymic lymphoma
T lymphocyte
tohate
track detector
transfection
translation
treatment
unequal crossover
ventilation scan
Th1/Th2 balance

irradiation system vitamine E
Japanese wall material
Japanese public whole-body counting
keratinocyte whole body irradiation
Ku p70 whole skeleton
Ku p80/XRCC5 wine
Lactobacilus casei wister rat
LCL wooden house
lead content xpg
lean body mass

ORGANIZATION AND STAFF

Division of Radiation Research

Yoshikazu Kumamoto, Ph.D., Director Mitsue Takeshita, Secretary **Fundamental Science**

Takehiro Tomitani, Ph.D., Head Sadao Shibata B.S.
Susumu Kinpara, Ph.D.

Radiation Protection

Yoshikazu Kumamoto, Ph.D., Head* Akihiro Shiragai, B.S.
Yutaka Noda, B.S. Tadashi Miura, D.D.S.++
Kazuo Iwai, D.D.S.++ **Bic-Emission**

Mikio Yamamoto, Ph.D., Head Nobuo Fukuda, M.D., Ph.D.++++ Mieko Kurano, B.S.
Tomoko Kokado, B.S.
Masahiko Hirasawa, M.Sc.+++++ Kimiko Kawano, B.S.+++++ Yoshio Machi, Ph.D.+++++ Nakahiro Yasuda, Ph.D.+++
Keichi Nishikawa, B.S.++
Koichi Ogura, Ph.D.++++ Hiroyuki Takahashi, M.F.++++ Kensuke Kishi, B.S.++ Hideyuki Kokubo, B.S.+++++ Junko Taniguchi, B.E.

Medical Information Processing Office

Kenjiro Fukuhisa, B.S., Head Toru Matsumoto, Ph.D.
shinichiro Sato, M.D., Ph.D. EIko Takeda

Medical Physics and Engineering Office

Masahiko Endo, Ph.D., Head Yuzuru Nakamura, Ph.D., Head Shigeo Furukawa
Hiroko Koyama-Ito, Ph.D. Shin-ichi Minohara, Ph.D.*
Nobuyuki Miyahara, Ph.D.*
Katsuyuki Nishimura, Ph.D.++++

Division of Radiation Medicine

Hirohiko Tsuji, M.D., Director **Section of Clinical Oncology** Shinroku Morita, M.D., Section Head Mizuho Sakurai, Secretary

1st Room

Tada-aki Miyamoto, M.D. Minoru Mukai, M.D. Tadashi Kamata, M.D.

Hideaki Sakaida, B.E. Yohji Kaizuka, M.E.++ Akira Watanabe, M.S.++ Tadayoshi Doke, Ph.D.++++

Medical Dosimetry

Takeshi Hiraoka, Ph.D., Head Akifumi Fukumura, B.S. kamane Omata, B.S. Suoh Sakata, Ph.D.++ Hidetaka Arimura, Ph.D.+ Hiroki Ohtani, B.S.++ **Senior Research Counselor** Eiichi Tanaka, Ph.D. katsuhiro Kawashima, Ph.D. Norimasa Nohara, Ph.D.

Division of Radiobiology and Biodosimetry

Isamu Hayata, Ph.D., Director Takeko Odaka, Secretary **Proliferation and Differentiation Mechanisms**

Eiichi Kojima, Ph.D., Head Kiyomi Eguchi-Kasai, Ph.D. Kaoru Tanaka, B.S. **Molecular and Cell Biology** Harumi Ohyama, Ph.D., Head Hiromi Itsukaichi

Masahiro Murakami, Ph.D. Masahiko Mori, Ph.D. Kumiko Fukutsu, B.S.* Bing Wang, M.D.++ Takashi Shimokawa++ Isao Shibuya, B.S.++

Shigenari Hashimoto, B.S.++ Tadashi Matsushima, M.S.++ **Cytogenetics and Cytometry** Isamu Hayata, Ph.D., Head* Masako Minamihisamatsu, B.S. Akira Furukawa, M.S.

Reiko Kanda, Ph.D.* Hiroshi Ohara, Ph.D.* Hiroshi Kato, M.D. Hisayuki Aoyagi, M.D. **2nd Room** Junetsu Mizoe, M.D. Takashi Nakano, M.D. Shosuke Matsuoka, M.D. Atsuko Terahara, M.D.

Atsuko Abe, M.D. and 2 Residential Doctors

Clinical Radio-Technology

Kunio Sakashita and 10 Staff Members

Section of Clinical Diagnosis Atsuo Akanuma, M.D., Section Head **Imaging Diagnosis**

Susumu Kandatsu, M.D. Masakuni Kanai, M.D. Masahisa Koga, M.D.

Kyosan Kan, M.D. and 1 Residential Doctor

Laboratory Examinations

Katsushi, Kurosu, M.D.

Hiroko Moriya and 4 Staff Members

Section of Nursing

Mitsuko Matsuda, Section Head Setsuko Fujimori

Etsuko Okazaki Fusako Kitane

Chiemi Murakami and 31 Staff Members with 4 Assistants

Pharmacy

Shin Watanabe and Hisashi Teshigawara

Division of Radiation Health

Atsuo Akanuma, M.D., Director

Clinical Hematology Makoto Akashi, M.D., Head Sastoshi Shiomomura, M.D. Yoshiko Kawase, B.S.

Misao Hachiya, B.S. Yoshiaki Osawa, B.S.++ **Clinical Immunology** Gen Suzuki, M.D., Head Masako Nose, B.S. Akiko Uzawa, B.S.

Senior Research Counselor Takaaki Ishihara, Ph.D. Takesaburo Mori, M.D.
Koki Sato, M.D.
Division of Biology and Oncology
koichi Tatsumi, M.D., Ph.D., Director **Cellular and Molecular Responses to Radiation**
Osami Yukawa, Ph.D., Head Sachiko Ichimura, Ph.D. Chidori Muraiso, B.S. Tetsuo Nakajima, M.S. Mitsuru Nenoi, Ph.D.
DNA Metabolism and Mutagenesis
Mitsuo Zama, Ph.D., Head Ikuko Furuno-Fukushi, Ph.D. Masahiko Takahagi, Ph.D.
Yuko Houki-Fujimori, B.Ph.+++++
Development Biology
Koichi Tatsumi, M.D.Ph.D.,Head* Yuji Ishikawa, Ph.D.
Tomohisa Hirobe, Ph.D.
Immunology and Molecular Oncology
Masahiro Muto, Ph.D., Head Shiro Aizawa, Ph.D.
Eiko Kubo Hitoko Kamisaku
Akira Fujimori, M.D., Ph.D. Ryutaro Fukumura+++++
Susceptibility to Radiogenic Cancer Kochi Tatsumi, M.D., Ph.D., Head Hisako Sakiyama, M.D., Ph.D.
Takeshi Furuse, Ph.D. Kazuko Yoshida, Ph.D.
Masumi Abe, Ph.D. Yuko Noda
Ryoko Araki, M.D., Ph.D.+++ **Senior Research Counselor** Toshihiro Sado, Ph.D.
Tamiko Iwasaki, M.D.
Yoshiro Aoki, M.D.* **Radiation Injury Control** Atsuo Akanuma, M.D.* Yasushi Yamamoto, M.D. Nobuyoshi Kobayashi, M.D.
Division of Accelarator Physics and Engineering
Kiyomitsu Kawachi, Ph.D., Director **Low Energy Beam Research** Yukio Sato, Ph.D., Section Head Atsushi Kitagawa, Ph.D.
Takanobu Yamada, B.S.* Takeshi Murakami, Ph.D.* Kazutoshi Suzuki, Ph.D.* Jun Yoshizawa, M.E.++++ Hirotsugu Owaga, B.S.*+
High Energy Beam Research Satoru Yamada, Ph.D., Section Head Masayuki Kumada, M.S.
Mitsutaka Kanazawa, Ph.D. Koji Noda, Ph.D.
Eiichi Takada, Ph.D.*
Masako Hosaka, Ph.D.+++++ Akira Mizobuchi, Ph.D.++++ Satoru Mitsumoto, Ph.D.++++ Kuninori Endo, Ph.D.++++ Tetsumi Tanabe, Ph.D.++++ Natsuji Araki, Ph.D.++ **Medical Physics Research** Tatsuaki Kanai, Ph.D., Section Head Masami Torikoshi, Ph.D.
Shinici Minohara, Ph.D. Nobuyuki Miyahara, Ph.D.
Naruhiro Matsufuji, M.E. Itsuro Tamanoi, Ph.D.++++ Hiromi Tomura, Ph.D.+++ Yasuyuki Futami, Ph.D.+++ Masahiro Ecdo, Ph.D.* Hiroko Koyama-Ito, Ph.D.* Akifumi Fukumura, B.S.* Fumio Nagasawa*

Hiroshi Tanooka, M.D. Ph.D. Norikazu Yasuda, M.D.

Division of Radiotoxicology

Jiro Inaba, Ph.D., Director **Metabolism and Decorporation** Sentaro Takahashi, Ph.D., Head Hiroshi Sato, Ph.D.

Yoshihisa Kubota, D.V.M. Chihiro Koshimoto, Ph.D.+

Dosimetry of Internal Exposure Nobuhito Ishigure, Ph.D., Head Takashi Nakano, Ph.D.

Hiroko Enomoto

Experimental Pathology

Yoichi Ogiso, D.V.M., Ph.D., Head Satoshi Fukuda, D.V.M., Ph.D. Yutaka Yamada, D.V.M., Ph.D. Haruzo Iida

Safely Engineering

Akira Koizumi, B.S., Head* Yuji Yamada, Ph.D.

Katsuhiko Miyamoto **Senior Counselor**

Osamu Matsuoka, Ph.D.

Division of Environmental Health

Masafumi Uchiyama, Ph.D., Director Kazuko Kodaira, Secretary

Source and Dosimetry of Natural Raidiation

Masafumi Uchiyama, Ph.D., Head* Masahide Furukawa, Ph.D.

Shinji Tokonami, Ph.D. Kenzo Fujimoto, Ph.D.*

Internal Radiation Dose Assessment

Yoshikazu Nishimura, D.V.M., Ph.D., Head

Shozo Hongo, B.S.* Masae Yukawa, Ph.D.

Yoshito Watanabe, Ph.D.

YOshikazu Kumamoto, Ph.D.* Koichi Ando, D.D.S., Ph.D.* Yuzuru Nakamura, M.D.* Junetsu Mizoe, M.D.* Shinroku Morita, M.D.*

Kunio Sakashita*

Division of Planning and Coordination

Fuminori Soga, Ph.D., Director

Tohru Nakahara, Section Head and 15 Staff Members

Division of Administration

Kimihiko Oda, Director

Section of General Affairs

Isamu Sato, Section Head and 14 Staff Members

Section of Accounting

Kazuo Hosokawa, Section Head and 17 Staff Members

Labolatory for Radioecology

Kiyoshi Nakamura, M.S., Laboratory Director

Section of Administrative Services Yoshio Hasegawa, Section Head and 5 Staff Members

Geochemical Rersearch

Shigeki Hirano, Ph.D., Section Head Masatoshi Yamada, Ph.D.

Tatsuo Aono, Ph.D. Jing Zhang, Ph.D.++

Bio-concentration Research

Ryoichi Nakamura, B.S., Section Head Motokazu Nakahara, B.S.

Toshiaki, Ishii, B.S. Mitsue Matsuba

Assessment of the Impacts of Environmental Radioactivity Teruhisa Watabe, M.S., Section Head

Setsuo Yokosuka

Environmental Transfer and Biokinetics

Masafumi Uchiyama, Ph.D., Head* Yoshikazu Inoue, M.S.**

Hiroshi Takeda, Ph.D. Kiriko Imai, Ph.D.*

Shoichi Fuma, M.S.

Chemical Behavior of Radionuclides

Masafumi Uchiyama, Ph.D., Head* Katsumi Kurotani, Ph.D.

Hidenori Yonehara, Ph.D. Kiyoko Imai, Ph.D.*

Sadao Shibata, B.S. Hiroshi Takeshita, B.S.

Senior Research Counselor Masaharu Okano, Ph.D. Tetsuo Iwakura, B.S.

Kazuo Watari, Ph.D. Kazuhisa Komura, Ph.D.+

Masahiro Wada, Ph.D.+

Jia Ming Hong, Ph.D.+(STA fellow) Pan Jia Lin+(STA fellow)

Izuru Kakuta, Ph.D.+ Shiro Sakurai, Ph.D.+

Yoshihiro Ikeuchi+ Kiyoshi Kitamura+

Yoshiyuki Taira+ Kiyomasa Ishikawa+ Yohei Koshima+

Division of Technical Services

Tatsuo Kondo, Director

Section of Thchnical Maintenance Nobuyasu Suzuki, B.S., Section Head and 7 Staff Members

Safety Promotion Office

Tsuneo Komata, Chief and 12 Stadd Members

Conputer Center

Shozo Hongo, B.S., Chief

Eiko Takeda, and 1 Staff Member

Service for Radiotoxicology

Space and Particle Radiation Science Research Group

Kazunobu Fujitaka, Ph.D., Supervising Researcher

Yutaka Noda*

Masahide Furukawa, Ph.D.* Kiyomi Eguchi-Kasai, Ph.D.*

Satoshi Fukuda, D.V.M., Ph.D.* **Experimental Radiotherapy**

Koichi Ando, D.D.S., Ph.D., D.M.Sc., Subgroup Leader

Sachiko Koike Kumie Nojima Hiromi Kanzaki Eriko Sato

kazumi Nakada

Perticle Radiobiology

Kazunobu Fujitaka, Ph.D., Subgroup Leader* Yoshiya Furusawa, Ph.D.

Masao Suzuki, Ph.D. Keiko Sekita

Space Radiation Environment

Hiroshi Yamaguchi, Ph.D., Subgroup Leader Hiroshi Yasuda, Ph.D.

Masashi Takada, M.S. Tetsuya Sakashita, M.S.++ Yukio Uchihori, Ph.D.+++ Tadayoshi Doke, Ph.D.+ Kazuko Kaiho

Michiko Takakura

Radiation Biophysics

Shinji Matsumoto, Ph.D., Subgroup Leader Bunshiro Goto

Environmental and Toxicological Sciences Research Group

Yuji Nakamura, Ph.D., Supervising Researcher

Methodology Development Yuji Nakamura, Ph.D., Head

Masahiro Doi, Ph.D.

Tetsuya Sakashita, Ph.D.++

Akira Koizumi, B.S., Chief
Satoshi Fukuda, D.V.M., Ph.D.* and 4 Staff Members
Section of Radiation Safety
Hajime Sato, Section Head and 12 Staff Members
Section of Laboratory Animal and Plant Husbandry
Torazo Sato, Section Head
Breeding
Satoru Matsushita, D.V.M., Ph.D. Akihiro Kawano, D.V.M., and 6 Staff Members
Laboratory of Veterinary Pathology and Microbiology
Junji Yamagiwa, D.V.M., Chief Chizuko Naruke

Laboratory of Animal Development
Masayuki Kitazume, D.V.M., Chief Masanori Okamoto, Ph.D.
Naomi Nakagata, M.D.++++
Training School
Michiko Abe, Ph.D., Head
Scientific Office
Hisamasa Joshima, Ph.D., Head Kiyoko Imai, Ph.D.
Mitsuru Nenoi, Ph.D. Susumu Kinpara, Ph.D.

Administrative Office
Tatsuo Fuchigami, B.S. and 2 Staff Members
Bioregulation Research Group
Toshihiko Ozawa, Ph.D., Supervising Researcher
Michiyo Takahashi, Secretary **Bioradical Research** Toshihiko Ozawa, Ph.D., Head*
Yoshie Shimazu, B.S.
Jun-ichi Ueda, Ph.D. Kazunori Anzai, Ph.D.

Biogeochemical Research Yasuyuki Muramatsu, Ph.D., Head Shigeo Uchida, Ph.D.
Satoshi Yoshida, Ph.D. Keiko Tagami, Ph.D. Tadaaki Bannai, M.S.** **Model Ecosystem Studies** Hiroshi Takeda, Ph.D., Head Kiriko Miyamoto, Ph.D.
Kei Yanagisawa, Ph.D. Shoichi Fuma, M.S.
Yoshito Watanabe, Ph.D.** Yoshikazu Inoue, M.S.**
Environmental Toxicology Sentaro Takahashi, Ph.D., Head Yoshihisa Kubota, D.V.M. Hiroshi Sato, Ph.D.*
X.Z.Sun, Ph.D.++(Domestic Research Fellow) Preethi Soysa, Ph.D.++(STA Exchange Scientist--1996/10/2-1997/1/6)
Senior Research Counselor
Tetsuo Iwakura, M.S.
Zen-ichiro Kawabata, Ph.D.++++
Low Dose Radiation Risk and Carcinogenesis Research Group
Toshiaki Ogiu, M.D., Supervising Researcher*
Low Dose Radiation Rersearch Shunsaku Sasaki, Ph.D., Head **Experimental Carcinogenesis** Toshiaki Ogiu, M.D., Head* Hiroko Ishii-Ohba, Ph.D.
Yoshiya Shimada, Ph.D. Mayumi Nishimura
Shigeru Kobayashi, M.D.+++++
Cancer Genetics
Hideo Tsuji, Ph.D.
Fumiaki Watanabe, Ph.D.+++ Chiharu Nishimori, M.S.***
Division of Advanced Technology for Medical Imaging

Yuri Miura, Ph.D.+++ Hideo Utsumi, Ph.D.++++ Shigeru Ono, Ph.D.++ Takashi Katsu, Ph.D.+ Yasuyuki Futatsugi, Ph.D.+

Expression of Bioregulator Genes Kazuko Tsuneoka, Ph.D., Head Hiroshi Ichihara, Ph.D. Masako Furuse Izumi Tanaka

Cytoregulation Hiroshi Inano, Ph.D., Head Keiko Suzuki, Ph.D. Makoto Onoda, Ph.D.

Explosive Study of Bioregulation Substances Nobuo Ikota, Ph.D. Head Hiroko Hama-Inaba, Ph.D. Hidehiko Nakagawa, Ph.D.

Senior Research Counselor Mikio Shikita, Ph.D. Akira Hanaki, Ph.D.

Genome Research Group

Tada-aki Hori, Ph.D., Supervising Researcher

Genome Sequencing and Informatics

Kazuei Mita, Ph.D., Head Kimihiko Sugaya, Ph.D.

Genome Analysis on Model Organisms Mitsuoki Morimyo, Ph.D., Head Tomoyasu Higashi, M.S. Etsuko Hongo

Human and Mouse Genome Mapping

Tada-aki Hori, Ph.D., Head Masashi Sagara, Ph.D. YOichi Matsuda, Ph.D. Toshiyuki Saito, Ph.D. Takashi Imai, Ph.D. Hiroko Ito

Human and Mouse Function

Kozo Morita, M.D., Director Taiko Joshima, Secretary

Imaging Physics and Tracer Chemistry

Kazutoshi Suzuki, Ph.D. Head Hideo Murayama, Ph.D. Terushi Haradahira, Ph.D. Shigeru Akiba Hinako Toyama, Ph.D.***** Hiroyuki Shinohara, Ph.D.***** Yuko Amou, Ph.D.*****

Eiichi Tanaka, Ph.D.*** Norimasa Nohara, Ph.D.*** Motoji Sasaki, M.S.** Masahiro Sasaki** Kotaro Nagatsu** Akihiro Ikemoto, M.S.** Hidekazu Kawashima** Yuichi Kimura, Ph.D.** Tomoyuki Hasegawa, Ph.D.**

Radiopharmaceuticals and Radiopharmacology Toshiaki Irie, Ph.D. Kiyoshi Fukushi, M.S. Masaomi Iyo, M.D.**** Hiroki Nanba, M.D.** Hitoshi Shinotou, M.D.**

Clinical Imaging

Katsuya Yoshida, M.D., Head Toru Matsumoto, Ph.D. Tetsuya Suhara, M.D. Yukio Tateno, M.D.*** Takeshi Iinuma, Ph.D.*** Nobuo Fukuda, M.D.*** Hiroshi Fukuda, M.D.*** Fumio Shishido, M.D.* Yasuhiko Sudo, M.D.** Akira Takami, M.D.** Yoshiro Okubo, M.D.** Hiroyuki Tadokoro, M.D.** Kazuhiro Shimada, M.D.** Shinobu Kitsukawa, M.D.** Akiyo Aotsuka, M.D.** Yasunori Imai, M.D.**

Tetsuya Saeki, Ph.D., Head Hideo Tsuji, Ph.D. masatake Yamauchi, Ph.D. Satsuki Tsuji

Mouse Gene Targeting Tadahiro Shiomi, Ph.D., Head Yoshinobu Harada, Ph.D. Manabu Koike, Ph.D.

Space and Particle Radiaton Science Research Group

Kazunobu Fujitaka, Ph.D., Supervising Researcher

Yutaka Noda, B.S.* Masahide Furukawa, Ph.D.* Kiyomi Eguchi-Kasai, Ph.D.* Satoshi Fukuda, D.V.M., Ph.D.* Junji Yamagiwa, D.V.M., Ph.D.* Satoru Matsushita. D.V.M., Ph.D.* Akihiko Kawano, D.V.M., M.S.* **Experimental Radiotherapy**

Koichi Ando, D.D.S., Ph.D.,D.M.Sc., Head Kiyoko Imai, Ph.D.

Sachiko Koike, B.S. Kumie Nojima, B.H.S

Particle Radibiology

Kazunobu Fujitaka, Ph.D., Head* Yoshiya Furusawa, Ph.D.

Masao Suzuki, Ph.D.

Space Radiation Environment Hiroshi Yamaguchi, Ph.D., Head Hiroshi Yasuda, Ph.D.

Masashi Takada, M.S. Tetsuya Sakashita, M.S.++ Tadayoshi Doke, Ph.D.++++ Miroslav Pinak, Ph.D.++ **Radiation Biophysics**

Shinji Matsumoto, Ph.D., Head

Safety Analysis Unit

Koki Sato, M.D., Director*

Kenzo Fujimoto, Ph.D., Section Head Yuji Nakamura, Ph.D., Section Head*

Toshiharu Himi, M.D.** Masato Asahina, M.D.** Yoshibumi Nakashima, M.D.** Yasuyoshi Hijikata, M.D.** Ohmi Terasaki, M.D.** Nozomi Akanuma, M.D.** Tsuyoshi Sasa, M.D.** Yasuhiro Someya, M.D.** Junichi Senda, M.D., Ph.D.** Maki Sakai**

Yoshihisa Akiyama, Ph.D.** Takayuki Obata, M.D.** Mika Yamaguchi, M.D.**

Division of Education and Sientific Services

Masayuki Tezuka, Director

Administrative Office

Mitsuru Kotoda, Head and 1 Staff Members

Education and Training

Hisamasa Joshima, Ph.D., Head

Mitsuru Nenoi, Ph.D.* Susumu Kinpara, Ph.D.*

Infoformation Network Promotion

Shozo Hongo, B.S., Head

Hiroshi Takeshita, B.S. and 3 Staff Members Kenjiro Fukuhisa, B.S.*

Eiko Takeda* Hitoshi Imazeki*

Laboratory Animal and Plant Sciences

Tsuneya Matsumoto, Ph.D., Head Junji Yamagiwa, D.V.M., Ph.D. Masayuki Kitazume, D.V.M. Masanori Okamoto, Ph.D.

Akihiro Kawano,D.V.M. and 6 Staff Members Naomi Nakagata, Ph.D.++++

Hiroshi Suzuki, Ph.D.+ Fumiaki Sato, Ph.D.+++++

Division of Human Radiation Environment

Kanae Nishizawa, Ph.D. Reiko Kanda, Ph.D.
Tetsuo Ishikawa, B.S. Shinji Yoshinaga, M.S.
Director of Special Research
Hiroshi Ohtsu, Ph.D.
Research Center of Charged Particle Therapy
Yasuhito Sasaki, Ph.D., Director General
Section of Administrative Services Hiyoshi Sato, Section Head and 12 Staff Members
Section of Accelerator Operation Satoru Yamada, Ph.D., Section Head* Takanobu Yamada, B.S.
Kazutoshi Suzuki, Ph.D.* Yoshikazu Kumamoto, Ph.D.*
Yukio Sato, Ph.D., Head and 6 Staff Members
Accelerator Operation Office
Eiichi Takada, Ph.D., Head and 3 Staff Members
Technical Management Office Takeshi Murakami, Ph.D., Head and 1 Staff Member
HIMAC Machine TIme Coordinator
Yoshiya Furusawa, Ph.D.* Koji Noda, Ph.D.*
Akifumi Fukumura, Ph.D.*

Masafumi Uchiyama., Ph.D., Director **Environmental Radiation Exposure** Kenzo Fujimoto, Ph.D., Head
Masahide Furukawa, Ph.D.
Shinji Tokonami, Ph.D. Takeshi Iimoto, Ph.D.++++
Biokinetics and Internal Dose Assessment Yoshikazu Nishimura, D.V.M., Ph.D., Head Masae Yukawa, Ph.D.
Yoshito Watanabe, Ph.D. Shozo Hongo, B.S.*
Internal Exposure and Human Models
Hisao Kawamura, B.S., Head Kunio Shiraishi, Ph.D.
Radiation Exposure Mitigation Masafumi Uchiyama, Ph.D., Head* Katsumi Kurotaki, Ph.D.
Hidenori Yonehara, Ph.D. Sarata Kumar Sahoo, Ph.D.
Kiyoko Imai, Ph.D.
Hiroshi Takeshita, B.S.* Sadao Shibata, B.S.*
Population Dose Assessment Kanae Nishizawa, Ph.D., Head Masaki Matsumoto, M.S. Tetsuo Ishikawa, B.S.
Epidemiology and Risk Assessment Yasuhiko Yoshimoto, Ph.D., Head Reiko Kanda, Ph.D.
Shinji Yoshinaga, M.S. **Senior Research Counselor**
Masaharu Okano, Ph.D. Tetsuo Iwakura, B.S.
Kazuo Watari, Ph.D. Sadayoshi Kobayashi, Ph.D. Gi-ichiro Tanaka, Ph.D. **Visiting Scientist**
Mantha Venkata Ramalinga Prasad, Ph.D.+ (STA fellow)
Kanitha Srisukwad, Ph.D.+(STA fellow) Young-ho Kim+

*Dual Capacity

**On leave of absense

+Visiting Scientist

++Research Fellow

+++National Institute Post Doctoral Fellow

++++Guest Scientist

+++++Guest Research Associate

a Higher Contents