1. PHYSICS

1．1. Measurements of Neutron Dose Equivalents and Characteristic of Thermoluminescent Dosemeter with Heavy Ions

Yoshikazu Kumamoto, Akihiro Shiragai, Yutaka Noda, Tatsuaki Kanai, Yukio Sato and Takeshi Murakami **Keywords:** radiation protection, shielding, neutrons, rem-counter, thermoluminescent dosemeter, LET

For radiation protection purposes, the dose equivalent behind the concrete shielding and the characteristic of the thermoluminescent dosemeter were measured with heavy ions.

To measure neutron dose equivalent, an ordinary Anderson-Braun rem-counter and a modified rem-counter were used. To compensate for the low sensitivity of the former in the neutron energy range above 20 MeV, C.Birattari et al. modified the counter, by introducing a lead energy moderator. This counter has a response similar to the fluence-to-dose equivalent conversion coefficient for neutrons of up to 1000 MeV. The neutrons were produced by bombarding a copper target with He, C, He and Si ions having energies from 100 to 800 MeV／n. The measurements were carried out behind shielding of 380 cm of concrete plus 50 cm of iron. As shown in fig.1, the difference in dose equivalents between the ordinary and modified rem-counters increases with increasing ion energies, indicating a large component of high energy neutrons. Also the dose difference between carbon and neon bombardment is small when the ion energy per nucleon is the same, although their atomic numbers differ.

For the absorbed dose measurement of organ doses in patients, the characteristic of the thermoluminescent dosemeter, Mg_2SiO_4:Tb., was studied with the irradiation of 60Co gamma-rays and carbon ions of 13.3 keV／μ and 75 keV／μ. As shown in Fig.2, for all radiations, supralinearity is found; the degree decreases with increasing LET. To investigate the radiation damage of the present dosemeter, 5 irradiations of 5 Gy were made with carbon ions of 13 keV／μ and 194 keV／μ. The sensitivity change was measured with the irradiation of 0.1 Gy of 60Co gamma-rays by repeating the irradiation and annealing. No radiation damages are found.

Fig.1 The neutron dose equivalents per h and per 10^8 particles per second behind the shielding of 380 cm of concrete and 50 cm of iron. Black symbols show measurements with an ordinary Anderson-Braun rem-counter. Open symbols show those with the modified rem-counter.

Fig.2 The thermoluminescent response of Mg_2SiO_4:Tb for 60Co gamma-rays, and 13.3 keV／μ and 75 keV／μ carbon ions.

1. PHYSICS

1．2. A Model of Optical Reflection on a Random Rough Surface and Its Applications to Monte Carlo Simulation of Light Transport

Takehiro Tomitani

Keywords : random rough surface, optical reflection, Monte Carlo simulation

The light transport problem is important in designing nuclear radiation detector systems that use scintillation or Cherenkov radiation. Reflection of light o■n a surface can be calculated accurately only when the surface is mirror-■■reflective. The other extreme case that is mathematically manageable is the one covered with extremely fine grains of reflective materials. The incident light rays are reflected or refracted among grains many times so that they lose the history of incidence and merge from the surface as though they are emitted from the light source of a rough surface and Lambert's law of luminance holds. All existing Monte Carlo codes are based on the Lambertian model of reflection and their applicability is certainly limited. P. Beckmann introduced a theory of reflection for electromagnetic waves on a random rough surface that is applicable to the reflection of optical light. According to Beckmann's theory, the power of the reflected light on the random rough surface can be approximately expressed in the following expression. Here we assume that the light of wave number, k, with zenith angle, ψ, is incident in the x-direction, and θ is the zenith; is the azimuth angle of reflected light; respectively; k Z = k cos θ and k x y = k sin θ indicate the vector component of the reflected light normal to the surface and that on the surface, respectively; F indicates form factori A indicates the surface element; σ and τ indicate the standard deviation of a normal distribution of roughness and the correlation distance, respectively, which define the statistical characteristics of the surface roughness. The reflection characteristic can be changed from mirror-reflection to Lambertian reflection by adjusting the ratio of parameters, τ／σ. To test the validity and applicability of Beckmann's model, a Monte Carlo simulation program for the light transport was developed. The program was tested with a simple model that simulates a gaseous scintillation counter consisting of a parallelepiped gas container viewed by a photomultiplier(PMT). The number of photons detected by the PMT was recorded as a function of time. The resultant time spectra are shown in Fig.1. The time characteristics of the light output vary almost continuously from mirror-reflection to Lambertian reflection as the ratio, τ／σ, decreases.

The proposed light reflection model interconnect the mirror-reflection model and the Lambertian reflection model and is useful for simulation studies of light transport.

[Publications]

Tomitani, T.: IEEE Trans. Nucl. Sci., NS-43(3), 1544-1548, 1996.Fig.1 Time spectra calculated by Monte Carlo simulation of photon transport inside the parallelepiped scintillator. The light source is located at the center of the counter.The parameters attached to the curves are explained in the text.

1. PHYSICS

1．3. Measurements of Metabolic Rate of 11C Auto Activity Induced from 12C Beams in Rabbit Thigh Muscle

Takehiro Tomitani, Mitsutaka Kanazawa, Kyosan Yoshikawa, Katsuya Yoshida, Tatsuaki Kanai and Yasuhiro Wada＊ (＊ Siemens-Asahi Medical Technologies Ltd.)

Keywords: heavy ions, fragmentation, auto activation, PET, metabolism, biological half-life

Treatment planning of heavy ion therapy is based on the range estimation in the medium. The range of the heavy ion is estimated from the CT number by look-up in a precalibrated conversion table. The range is proportional to the electron density of the medium, while CT number is not only dependent on the electron density, but also the atomic number of the medium.
Therefore some kind of experimental checking means is needed. Positron emitting fragments, 11C, induced by the projectile fragmentation process during 12C beam therapy privides useful information for estimating the end point distribution of primary particles.

The induced activity may be metabolized inside the human body. To image the 11C distribution, metabolism must be stable during the measurements. The induced activity distributes uniformly irrespective of the organ species. The activity induced in blood vessels will rapidly circulate over the whole body, while that stopped inside the tissues will be metabolized relatively slowly. Prior to clinical measurements, we performed the metabolic rate measurements on animals. To this end, the organ must be uniform and large enough to stop 11C beams within it. Rabbits were chosen since they are easy to anesthetize. Thigh muscle was chosen since its size is large enough for the experiments and the identification of its shape and position can be easily done outside the body, while brain was excluded because of its shape and liver was excluded because of the difficulty in identifying its shape and position externally.

A rabbit was first anesthetized, fixed to a wooden board and then irradiated with 12C beam collimated with a 6 cm thick brass slab having a hole of 2 cm in diameter. The rabbit was transferred from irradiation vault to the PET within 5 minutes which is sufficient enough to cool down positron emitters of short half-lives except 11C. 11C activity distribution was measured in the dynamic mode for 60 minutes. One example of an induced activity distribution is shown in Fig.1 along with the transmission image of the same section for comparison. After regression analysis of the dynamic study data, the biological half-lives were obtained as 68 min and 90 min for 12C irradiation of 10 Gy and 1.3 Gy, respectively. The activity curve inside the region of interest is shown in Fig.2 as a function of time along with the fitted curve and the curve corresponding to the physical half-life of 11C. These metabolic half-lives were longer than the physical half-life of 11C, 20.39 minutes, and confirms the measurement technique.

[Publications]

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

Kanazawa, M., Yoshikawa, K.,Yoshida, K., Wada, Y., Fukumura, A. and Kanai, T.: NIRS-M-116／HIMAC-013, 169-170, 1996.

Fig.1 Left photograph is the central section image of the 11C auto activity generated from 12C beams inside a rabbit thigh muscle. Beams were spread out in b

Fig.2 Time-activity curve of 11C in the rabbit thigh muscle.

1. PHYSICS

1．4. An Experiment on Remote Action against Man in Sense Shielding Condition

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

Keywords: qi-gong, tohate, sense shielding, suggestion, extrasensory

Some masters of qigong (Chinese traditional arts for health) can perform a tohate that makes an opponent step back rapidly without being touched. In this study, a master performed tohates for his pupil, when the master and pupil were in separate rooms, one on the 2nd floor, and the other on the 5th floor, of a building. The master's acting time and his pupil's response time were recorded separately. One trial consisted of 3 tohates being performed in 3 min at intervals of 20 s or more. A total of 16 trials were made.

Fig.1 illustrates the frequency distribution of time differences between the master's acting time and his pupil's response time of the tohates during 16 trials. Time differences of less than 1 s were observed 6 times (one time in each of 6 trials). The probability of the student stepping back during the 3 tohates in one trial within 1 s is 0.11. The probability that the event described above would accidentally occur in 6 trials or more among the 16 trials is 0.0058. This calculation may imply that all tohates do not depend on the master's suggestion and these is some unknown transmission of the master's acting, since the above result is significant for an approximate synchronous timing between acting and response.

Fig.2 shows an averaged amplitude topograph of α wave of the EEG of the qigong master at rest. There exists an area of higher amplitudes at the center of the frontal region. It is known that α wave amplitudes at the center of the frontal region increase, particularly when a subject is concentrating.

[Publications]

Yamamoto, M., Hirasawa, M., Kawano, K., Yasuda, N. and Furukawa, A.: J. Int. Soc. Life Information Science, 14, 97-101, 1996.

Fig.1 Frequency distribution of time differences between acting time and response time. Fig.2 Averaged amplitude topograph of α wave of the EEG of the qigong master at rest.

1. PHYSICS

1．5. Experiment on Unknown Subconscious Information Transfer with Auditory Brain Evoked Potentials

Masahiko Hirasawa and Mikio Yamamoto

Keywords: subconsciousness, extrasensory, information transfer, brain evoked potential, P2 peak latency

Warren et al. (J. Parapsycho., 56, pp.1-30, 1992) suggested man's extrasensory recognition, by means of a visual brain evoked potentials in their experiment. This reports an investigation on the possibility of extrasensory recognition through an experiment on brain evoked potentials generated by auditory stimuli. The subject listened to a sound pulse of around 630 Hz for 50 ms and his electroencephalogram was recorded for 1 s before and after each pulse at his right frontal region by applying the monopolar method with a reference electrode on the right earlobe.

Each trial was composed of 4 pulses at intervals of 3 s, and the subject tried to identify one target pulse which had been randomly determined from the 4 pulses by a computer just before the trial. The choice was made without the subject's knowledge. One hundred trials were repeated with the same subject. The subject was a healthy 50 year old man. Results of his guessed target are shown in Table 1. "p" indicates the probability of occurrence by chance of guesses that could have no less deviation than the table result has. Fig.1 shows a pair of curves of auditory brain evoked potentials that were obtained from averages of 98 targets or 98 non-targets. The magnitudes of the peaks, P1 to P2 are almost the same between the two curves, but differences in latencies of the peaks, P1 and P2 occur on them. The two-sample-test (t-test) was carried out for two sets of P2 peak latency data, one for the targets, the other for the non-targets. The results are shown in Table 2. The "p" indicates the probability of occurrence by chance of sampling which could give a bigger difference than that calculated from the average value for the latency data of the targets and that of the non-targets as shown in the table.

The result of conscious recognition by means of guessing targets was judged not to be significant at a 5% level of significance (one-tailed), which demonstrated that there is no extrasensory recognition in the subject's consciousness. However, the difference of the latencies of the peaks P2 of the auditory brain evoked potential curves calculated between targets and non-targets was judged significant at a 5% level of significance (one-tailed), which demonstrated that there may be an extrasensory recognition in the subject's subconsciousness.

[Publications]

Hirasawa, M. and Yamamoto, M. : J. Int. Soc. Life Information Science, 14, 32-37, 1996.

Fig.1 Auditory brainevoked two potential curves.

Each was obtained as the average of either 98 targets or 98 non-targets.

1. PHYSICS

1.6. Ion Recombination Loss and Polarity Effect for Very Small Ionization Chambers

Takeshi Hiraoka,Kaname Omata,Akifumi Fukumura and Mitsue Takeshita

Keywords: ionization chamber,saturation,ion recombination, polarity effect

With increasing use of linear accelerators for photon beam radiosurgery, many kinds of very small ionization chambers have been commercially developed. Ionization chambers are most commonly used for measurements of radiation dose. Ion recombination loss and polarity effect are the most important factors to express chamber characteristics. To investigate the characteristics, one parallel plate chamber and six cylindrical and two hemispherical ionization chambers, which had nominal volumes between 0.1ml and 3μl, were used.

The polarity effect is usually caused by the presence of extra cavities around the collecting lead of the chamber. For high energy photon radiations, the polarity effect is also caused by flow in or out of Compton electrons on the collecting lead. This largely depends on the volume of the collector and the insulator. Measurements were carried out using 70 MeV proton beams from the AVF cyclotron to check the effect caused by Compton electrons on the polarity effect. Dose rate was approximately 25Gy／min at the entrance plateau of the beam. Irradiations were made with a 6cm×6cm field in air.

Measurements were made using 10MV X-rays for saturation characteristics and the polarity effect. Irradiations were carried out with a 10cm×10cm field at the peak depth in a phantom and dose rate was 3Gy／min with the pulse repetition rate of 40pps.

Ionization charge was measured by Keithley model 617 electrometer for 10MV X-rays and for 60Co gamma rays, and a vibrating reed electrometer, Cary model 401 was used for 70MeV proton beams.

The polarity effect as a function of applied voltage was evaluated as the charge ratio for vertical irradiation of the 10MV X-ray beam. All the chambers shown that the effect were less than 5% except for one chamber which was larger than 50%. For the hemispherical chambers, irradiations were made for both vertical and horizontal directions. The value of the charge ratio differed between irradiation directions, however, the shape of the curve was quite similar.

Using high energy proton beams which are not stopped in the collecting electrode, are one of the best sources to check the polarity effect. For relatively high ion collection efficiency, the polarity effect is small for all chambers used. The polarity effect for 60Co gamma ray beam was measured at one point near the saturation voltage. From the results obtained for 60Co gamma ray and those for the proton beam, the effect did not change very much.

Ion collection efficiency is defined as the ratio of observed to produced ionization charges in a chamber. The ion collection efficiency as a function of applied voltage was measured for 10MV X-ray and 70MeV proton beam. The experimental points were shown as mean values for both polarities. In order to compare the values obtained for the different chambers, the equation given by Boag was used to get the equivalent gap length of each chamber. This was considered as theoretically equivalent to the parallel

plate shape. The experimental points of ion collection efficiency measured for all ionization chambers were plotted as a function of d^2/V, where d is the gap length and V the applied voltage. If the ionization density generated in each chamber gas is identical, the experimental values are ideally expressed as one line. The actual values differed for the chambers because their shapes deviated from ideal cylindrical or spherical shapes. It was concluded that the ion recombination correction factor for very small ionization chambers must be determined experimentally for the actual beam to be used.

1. PHYSICS

1.7. Dosimetry Intercomparison of Therapeutic Proton Beam

A. Fukumura, T. Hiraoka, K. Omata, M. Takeshita, K. Kawachi, T. Kanai, D. T. L. Jones 1, A. N. Schreuder 1, J. E. Symons 1, E. Pedroni 2, R. Ferrand 3 and A. G. Molokanov 4 (1 NAC, South Africa, 2 PSI, Switzerland, 3 CPO, France, 4 JINR, Russia)

Keywords: dosimetry intercomparison, proton beam, ionization chamber

The traceability for the absorbed dose of a proton beam has not been established by national standard laboratories so far. Therefore it is important for proton therapy facilities to compare the absorbed dose evaluated by their dosimetry system with others. In addition, dosimetry of the proton beam is applicable to dosimetry of a heavy ion beam, which is used for clinical trial at the HIMAC facility in our institute.We took part in the international proton dosimetry-intercomparison program, which was organized by the National Accelerator Centre (NAC) of South Africa and involved Paul Scherrer Institut (PSI) of Switzerland, Centre de Protonth' erapie d' Orsay (CPO) of France and the Joint Institute of Nuclear Research (JINR) of Russia.The detectors which each facility brought were air-filled ionization chambers with a tissue equivalent wall. The NIRS and PSI used the T2 type ionization chambers made by Exradin and CPO had IC18 type by Far West Technology. The JINR chamber corresponded to the T2 type. NAC had the Farmar type chamber, as well as T2 and IC18 chambers.To begin with, each chamber was calibrated in 60Co γ-ray standard field. Next, it was installed on the beam line in the water tank and irradiated with a proton beam of a preset quantity.Measurements of the proton beam were carried out under three conditions as follows:

1: 5 cm depth in water for the monoenergetic proton beam which has a range of 24 cm in water;

2: 19 cm depth in water for the proton beam which has a range of 24 cm in water and the spread-out Bragg Peak of 10 cm width in water;

3: 9 cm depth in water for the proton beam which has a range of 12 cm in water and the spread-out Bragg Peak of 6 cm width in water.

According to the European proton dosimetry protocol based on Bragg-Gray cavity theory (S. Vynckier, et al. , Radioth. Oncol., 20, 53, 1991; S. Vynckier, et al. , ibid., 32, 174, 1994), each facility individually evaluated the absorbed dose in water using charge collected during the irradiation and the cobalt calibration factor for its ionization chamber.

Table 1 summarizes the results of this dosimetry comparison. Each value is the ratio of the absorbed dose evaluated by each facility to the mean value. The JINR data were excluded here because its system could not measure collected charge with good accuracy. Good agreement is seen within about ± 1% variation for most conditions. This means that it is possible to standardize proton dosimetry internationally when the same protocol is adopted.

It should be noted that there is another protocol, mainly adopted in United States, in which the W-value of air for the proton beam is a few percent lower than that of the European protocol (AAPM Report 16, 1986). To establish the global traceability of proton dosimetry, more data on the W-value for a proton beam are required.

1. PHYSICS

1.8. Status of Ion Sources for HIMAC

A. Kitagawa, M. Muramatsu, H. Ogawa, Y. Sato, S. Yamada, J. Yoshizawa

Keywords: ion source, Penning, electron cyclotron resonance

A heavy-ion source for an accelerator is usually tuned so as to realize the maximum intensity of highly charged ions. However, the ion sources for the heavy-ion radiotherapy are required to have a steady beam performance, rather than the maximum intensity, i.e. reproducibility, including intensity and emittance, and easy operation at any time. In this purpose, HIMAC injector has two types of ion sources. One is a Penning ion source (PIGIS) for lighter ions and ions from solid materials.

The PIGIS is an indirectly heated-cathode type source. The arc power supply is operated in the pulse mode. Very low-duty pulsed operation realizes a very long source lifetime and high intensities for highly charged ions. The long-term stability of the beam intensity depends strongly on the voltage stability of the arc power supply against the varying cathode condition. This situation can be appreciably improved by adding a stabilizing system of the bombardment power instead of filament current. The arc voltage and the current were about 900 V and 4 A, respectively. The vacuum pressure was about $7\times10-6$ torr in the vacuum chamber; the pressure at the plasma was estimated to be over $10-4$ torr. The beam intensity was about 300 eμA. At present, carbon ions are obtained by sputtering a graphite block with a mixture of $N2$ and Ne gases. Since the sputtering technique reduces the amount of carbon deposition on the cathode insulators, the technique is very effective for extending the source lifetime. Other ions of solid materials can be produced using the same technique.

The ECRIS has a single closed ECR zone with 10 GHz microwaves. Plasma confinement is ensure by a simple minimum-B magnetic structure with two mirror coil magnets and a sextupole permanent magnet. The maximum axial mirror fields are 9.3 kG and 7.6 kG. The radial sextupole field is 8.0 kG on the chamber wall. An ECRIS is able to easily maintain stable operation.

Although the heat-up of the plasma chamber was the cause of fluctuation, it has almost been surmounted by an improvement in the cooling efficiency of the plasma chamber. The microwave power was about 700 W, and the pulse width was 5 ms. The afterglow peak was appeared after the microwave turn-off, but was not used because of the worse stability. The vacuum pressure in the plasma chamber was estimated to be around $2\times10-6$ torr. The extraction voltage was 24 kV. The beam intensity was about 340 eμA. The $CH4$ gas is used as ionized gas for the production of $C4+$, whereas $CO2$ gas is usually used for the $C2+$. It seems that the charge-state distribution depends on the amount of H or O ions which those play the role as a 'support gas'.

All of the devices of the accelerator components are made controllable through a computer system. All of the parameters and measured values can be saved as a parameter file after beam tuning. Under the well-known condition, since the parameter set up can be obtained automatically, it is only needed for an operator to select the parameter file and to turn on the start-up button.

The beam intensity varied during the first one hour, because the vacuum pressure in the plasma chamber

changed along with the heat-up of the chamber wall. After one hour, it was seemed that the intensity approached the value set by the given parameters without any tuning. A good reproducibility of the beam was obtained. The typical beam performance is summarized in Table 1.

The transmission of the injector included the charge-stripping efficiency located at the output end of the linac. The intensity of the sources was reduced for each requirement by a mesh-type attenuater. In a typical weekly schedule of beam time, on Monday, the whole accelerator facility is inspected and maintenance is carried out during the daytime; beam tuning begins in the evening.

Patients are treated during the daytime of other weekdays. Every night and on weekend, beam time is available for experiments. The accelerator facility is in operation from the evening of Monday to the night of Saturday, or the morning of Sunday. The fluctuation and drift of the intensity in the long-term stabiliby of the injector beam throughout a week are less than 10% and less than 2% during about 100 hours without any beam tuning.

[Publications]

A. Kitagawa, et al.:Rev. Sci. Instrum. 67(3), 962 (1996)

S. Shibuya et al.:Rev. Sci. Instrum. 67(3), 1171 (1996)

1. PHYSICS

1.9. The Very Low Ripple Synchrotron Power Supply for HIMAC

M. Kumada, S. Yamada, K. Noda, M. Kanazawa, N. Araki, S. Sato and E. Takada

Keywords: synchrotron power supply, ripple, harmonic content, common mode, beam spill

A need for slow extraction in synchrotron lead to make an effort of realizing a high power synchrotron power supply below a ppm level. On the other hand, high power converter have to adopt a thyristor rectified control system. It is also known, however, that this system inherently has rich noise source over wide range of frequency spectrum, especially known as thyristor spike noises in addition to harmoonic ripples. Major part of a history of R & D of the power supply of the synchrotron was to reduce this noise and ripples. In spite of this necessity of high ripple performance, the best power supplies known prior to our research have been around several ppm in a relative current ripple level and have never went below a level of ppm.

At HIMAC, a level around 0.1 ppm was achieved. This is realized through a detailed analysis of the source of the ripple and spike. The analysis took into account of the effect of the ground current where it is considered in the normal and common mode. For analytic treatment we established a formulation of the mode separation method. There, the string of magnets is treated as a ladder circuit, which is a circuit of series and parallel resonant circuit. The parallel resonance is treated by assuming stray cpacitances between coils and magnet yokes. We found this resonance is suppressed by bridge resistor which is shown in Figure 1.

Furthermore the ripple and the spike is made to be confined in the power supply system through a path of the voltage source of the grounded thyristor and the common mode low pass filter. Thus thyristor spikes and hige frequency ripples are removed by the static method, namely the bridge resistor and the low pass mode filter.

The additional source of 50 Hz and ripple were identified. 50Hz source due to a noise of a current sensor was removed. 100Hz source was suppressed by newly developed active filter. The performance is shown in Figure 2.

[Publications]

M. Kumada, PhD thesis, Graduate Univ. of Advanced Study, 1996, also, submitted to particle Accelerator.

Fig.1 Suppression effect of bridge resistor to resonanct ripple of magnetic field

Fig.2 Relative current ripple without, with HPF and with HPF＋BPFand with BPF. The ripples are normalized by a rated current.

2. CHEMISTRY

2．1． ESR Spin Trapping Studies on the Reactions of Hydroperoxides with Cu(■) Complex

Jun-ichi Ueda and Toshihiko Ozawa

Keywords: copper (■) complex, tert-butyl hydroperoxide, cumene hydroperoxide, ESR, spin trapping

Free radicals such as peroxyl (ROO·) and alkoxyl (RO·) radicals generated from lipid hydroperoxides with iron, have been recognized as mediators of tumor initiation and promotion. Copper has received less attention than iron, but is known to be more reactive than iron in stimulating the decomposition of hydrogen peroxide and hydroperoxide. Therefore, biological damage such as DNA damage, protein modification, and oxidation of low-density lipoproteins may be caused by the reactions of hydroperoxides with Cu(■) ion. Although Cu(■) ion exists as a complex in living organisms, there have been few reports about the reactions of Cu(■) complexes with hydroperoxides. Then, we studied the reactions of a Cu(■) complex, Cu(CyHH)2 {CyHH: cyclo(L-histidyl- L-histidyl)}, with lipid hydroperoxide model compounds such as tert-butyl hydroperoxide (tBuOOH) and cumene hydroperoxide (Cumene-OOH) using some spin traps.Fig.A) shows an ESR spectrum obtained from the reaction of Cu(CyHH)2 (0.25 mM) with tBuOOH (25 mM) in the presence of 5,5-dimethyl-1-pyrroline N-oxide (DMPO) (25 mM) at pH 7.4. This spectrum consists of two ESR signals. One with hyperfine splitting constants (hfsc) of aN(1)＝1.49 mT and aH(1)＝1.62 mT is assigned to the DMPO adduct of tert-butoxy radical (·OBut) based on close agreement to previously reported hfsc. The other [aN(1)＝1.44 mT, aH(1)＝1.05 mT and aH(1)＝0.14 mT] is assigned to the DMPO adduct of tert-butyl peroxyl radical (·OOBut), again based on the reported hfsc agreement. Fig.B) shows an ESR spectrum observed from the reaction of Cu(CyHH)2 (0.25 mM) with t BuOOH (25 mM) in the presence of PBN (25 mM) at pH 7.4. This ESR signal [aN(1)＝1.50 mT and aH(1)＝0.33 mT] is assignable to the PBN adduct of methoxy radical (OCH3·), because of the reported hfsc agreement. Fig9.C) shows an ESR spectrum obtained from the reaction of Cu(CyHH)2 (0.25 mM) with tBuOOH (25 mM) in the presence of POBN (25 mM) at pH 7.4. This ESR signal [aN(1)＝1.59 mT and aH(1)＝0.27 mT] is assignable to the POBN-methyl radical (CH3·) adduct because of the reported hfsc agreement. In order to ascertain the carbon-centered radical assignment, the water-soluble nitroso spin trap, 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS), was used. Figure D) shows an ESR spectrum obtained from the reaction of Cu(CyHH)2 (0.25 mM) with tBuOOH (25 mM) in the presence of DBNBS (12.5 mM) at pH 7.4. This spectrum consists of two ESR signals. One major signal [one nitrogen (aN(1)＝1.38 mT), three magnetically equivalent protons (aH(3)＝1.35 mT), and two magnetically equivalent meta-protons (aH(2)＝0.07 mT)] can be assigned to the DBNBS-CH3 adduct. Similar results are obtained from the reaction of Cu(CyHH)2 with Cumene-OOH. In conclusion, the formation of ROO· and RO· radicals from the reaction of tBuOOH or Cumene-OOH with Cu(CyHH)2 was confirmed by ESR using DMPO as a spin trap. On the other hand, neither ROO· nor RO· was observed when PBN, POBN, or DBNBS was used in place of DMPO. But, mainly CH3·, probably generated by β-scissions of RO·, was trapped by these spin traps. Rapid unimolecular decomposition of tert-butoxyl radical to CH3· and acetone is a well established

reaction.

[Publications]

1)Ueda, J., Shimazu, Y. and Ozawa, T.: Free Radic. Biol. Med., 18, 929-933, 1995. 2)Ueda, J., Hanaki, A. and Nakajima, T.: Chem. Pharm. Bull., 43, 359-361, 1995.

Fig9. ESR spectra obtained from the reactions of Cu(CyHH)2 (0.25 mM) with tBuOOH (25 mM) in the presence of (A) DMPO (25 mM), (B) PBN (25 mM), (C) PO

Instrumental conditions: microwave power, 10 mW; modulation amplitude, 0.079 mT; amplitude, 2x100; time constant, 0.03 s; scan time, 2 min. Spectra were reco

2. CHEMISTRY

2．2． Synthesis of (-)-Anisomycin Derivative from (S)-Pyroglutamic Acid Derivative

Nobuo Ikota

Keywords : polyhydroxylated pyrrolidine, (-)-anisomycin, antibiotic, (S)-pyroglutamic acid, cis-dihydroxylation, chiral ligand olyhydroxylated pyrrolidines show interesting biological activities such as glycosidase inhibitory activity. In continuation of our work on the synthesis of chiral polyhydroxylated amines, we describe here (Fig.1) the facile synthesis of (-)-anisomycin derivative(9) from (S)-pyroglutamic acid derivative. Dihydroxylation of trans-α,β-unsaturated methyl ester (2), prepared from 1 by hydrolysis with aqueous lithium hydroxide, methylation, and deselenenylation in 80% yield, with potassium osmate (0.04 equiv.) using hydroquinine 9-phenanthryl ether (0.15 equiv.) as a chiral ligand in the presence of $K_3Fe(CN)_6$ (3 equiv.) and K_2CO_3 (3 equiv.) in tert-BuOH-H_2O (1:1) at 0℃ for 24 h gave 3a and 4a in a ratio of 19:81 in 71% yield. The ratio of 3a and 4a was determined by high performance liquid chromatographic (hplc) analysis after their conversion of 3a and 4a into the coresponding diacetate (3b and 4b) (pyridine, acetic anhydride). The mixture of 3a and 4a was converted into the corresponding TBS ether (TBS chloride, imidazole, dimethylformamide (DMF)) and the diastereoisomers (3c and 4c) were separated by column chromatography. The major isomer (4c) was reduced with $LiBH_4$ in the presence of lithium triethylborohydride in ether to provide an alcohol(5), which was then converted to the pyrrolidine derivative (6a) via mesylate (MsCl, TEA, CH_2Cl_2; then tert- BuOK, THF) in 40% yield. The configuration of 6a was confirmed by converting it into the known pyrrolidine derivative (7). Thus, the removal of TBS group in 6a with tetrabutylammonium fluoride in THF followed by di-O-benzylation (NaH, DMF-THF, then BnBr) gave 6b in 65% yield. Cleavage of tert-butoxycarbonyl and trityl groups in 6b with acidic conditions (MeOH:10% HCl＝1:1, 70℃) followed by N-benzylation with benzyl bromide in the presence of K_2CO_3 in acetone gave 7 in 32% yield. Oxidation of 7 by the method of Swern followed by reaction with 4-methoxyphenylmagnesium bromide in ether gave 8 as the sole diastereomer, which was then treated with triethylsilane in the presence of trifluoroacetic acid and trifuluoromethansulfonic acid in CH_2Cl_2 to afford 8b in 31% yield. In this reaction, without addition of trifuluoromethansulfonic acid 8b was not obtained. N- Benzyloxycarbonyl-3,4-dihydroxy-2-(4-methoxyphenyl)pyrrolidine (9) was obtained in 60% yield after debenzylation of 8a (10% palladium carbon, 99% HCOOH, EtOH) followed by N-benzyloxycarbonylation. Compound 9 was easily converted into anisomycin in good yield.

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2. CHEMISTRY

2．3．Synthesis of Dithiocarbamate Derivatives and Spin Trapping of Nitric Oxide In Vivo with Their Iron Complexes

Hidehiko Nakagawa, Nobuo Ikota and Toshihiko Ozawa

Keywords: dithiocarbamate, iron complex, nitric oxide, ESR, spin trapping, L-proline

Nitric oxide (NO) is suggested to be an endogenous radical compound and to play an important role in inflammation, neurotransmission and vasodilation. A few iron complexes of dithiocarbamate derivatives have been used for spin trapping of nitric oxide both in vivo and in vitro. In this report, we describe the synthesis of a series of dithiocarbamate derivatives which have the L-proline moiety and the detection of nitric oxide in septic shock model mouse.A solution of L-proline in aqueous ammonia was mixed with a small excess of carbon disulfide in an equal volume of ethanol at 4℃, and then lyophilized to yield dithiocarboxy-L-proline (1, DTCP) as a slightly yellow powder. In the same manner, 4-trans-hydroxy-L-proline and 4-trans- (methoxymethyl)oxy-L-proline, which was derived from 4-trans-hydroxy-L-proline with methoxymetyl chloride, were converted to DTCHP (2) and DTCMP (3), respectively.For the preparation of iron complex (DTCX-iron complex), each dithiocarbamate was mixed with a half equivalent of ferrous sulfate in 40mM Tris-HCl (pH 7.4) under an anaerobic condition. The prepared complex solutions were anaerobically stored at 4℃ until use.Synthesized dithiocarbamate iron complexes were used to examine the trapping ability of nitric oxide in 40mM Tris buffer. The NO-adduct signals were detected by ESR spectroscopy and increased as a function of nitric oxide concentration. In these experiments, the three synthesized complexes were suggested to have almost the same affinity for nitric oxide.Septic shock model mice were obtained by a treatment of lipopolysaccharide (LPS, E.coli 026:B6) to the tail vein of ddY female mice. The complexes were injected into the septic shock model mice intravenously and a certain amount of blood was collected in a heparinized capillary 15 min after injection. The ESR spectra of the blood in the capillary were measured within 6 min.The relative amount of NO-adduct of each complex was measured at 3, 5 and 7 hours after LPS treatment. Using DTCP-iron and DTCMP-iron complexes as spin traps, NO-adducts were detected in mouse blood, and the adduct amounts increased with time after the LPS treatment. By contrast, NO-adducts of DTCHP-iron complex were detected only at noise level. The relative amount of detected NO-adducts was larger for DTCP-iron complex than DTCMP-iron complex. Although these complexes were suggested to have same affinity for nitric oxide in an in vitro experiment, it was shown that these complexes had different signal intensities from the NO-adducts in vivo. These results suggested that the complexes had different properties or behavior in the mouse body, and that they were distributed differently in the body and were trapped by nitric oxide in different places. The distribution and metabolism of these complexes or their NO-adducts in mice remain to be studied.

[Publications]

Fig.1 Structures of synthesized dithiocarbamate derivatives and their iron complexes.

3．1 BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．1 Studies on Neon Beam Induced DNA Double-Strand Breaks and Break Features which Depend on Chromatin Structure

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Keywords:DNA double-strand break, heavy ions, chromatin structure

The nature of DNA breaks induced by heavy ion beams with high LET may differ from those induced with X-rays. The main biological effects of heavy-ion radiation are thought to be mediated by DNA damage. Heavy-ion radiation deposits an energy more densely along its track than X-rays and this may cause unequal distribution of ionization in a cell. On the other hand, the distribution of DNA filaments in a cell changes as chromatin structure changes. We hypothesized that the quantity of DNA double- strand breaks induced by heavy-ion radiation may vary with the chromatin structure. DNA double-strand breaks induced by X-ray or neon beam irradiation in an ionizing radiation sensitive mouse mutant cell line (SL3-147) were examined before or after chromatin proteolysis. Because SL3-147 cells show DNA double-strand break repair deficiency, the DNA double-strand breaks were expected to remain unrepaired.Cells were exposed to X-rays from a Shimazu model Shinai X-ray Irradiator at 200kVp, 20mA, with 0.5mm aluminum and 0.5mm copper filtration, at NIRS, or they were exposed to neon beams from a cyclotron at the initial energy of 135 MeV／u, at RIKEN. Doses for the cell irradiation with X-rays were 30, 60 and 80 Gy. Doses with the neon beams were 30, 60 and 80 Gy and each dose was applied at the LET of 62.8 keV／μm or 205.5 keV／μm. The amount of DNA double-strand breaks for both beam irradiations was estimated by pulsed-field gel electrophoresis. There were no obvious differences in the size distribution of DNA fragments, based on electrophoresis analysis after the irradiations. One band (about 5.7 Mbp in size) and some smear bands occurred between 1 Mbp and 5.7 Mbp. The increase in the number of DNA double-strand breaks was dose dependent for both beam irradiations. We also analyzed the relation between chromatin structure and generation of DNA double-strand breaks by comparing the intact cell with the proteolyzed chromatin (naked DNA) irradiated with X-rays or neon beams. Both beam irradiations showed remarkable dose responses for the DNA double-strand breaks on naked DNA irradiation. The number of DNA double-strand breaks induced by X-ray or neon beam irradiations after proteinase K treatment was greater than that for non-treated cells. A decrease in the 5.7 Mbp DNA band was observed in concurrence with the increase in the relatively smaller smear bands between 5.7 Mbp and 1 Mbp.These results strongly suggest that destruction of the chromatin structure accounts for the hypersensitivity to X-rays and heavy-ionradiation after proteolysis treatment.

[Publications]

Murakami, M., Eguchi-Kasai, K., Sato, K., Minohara, S., Yatagai, F. and Kanai, T.: J. Radiat. Res., 36, 258-264, 1995

3．1　BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．2　The Role of DNA Repair on Cell Killing by Neutrons

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Keywords : Keywords: neutrons, DNA double-strand break, radiosensitive mutants

It has been suggested that initial double-strand break (dsb) correlates well with cell death induced by conventional radiations. However for high linear energy transfer (LET) radiations, values of relative biological effectiveness (RBE) for dsb induction were about 1 for particles with LET below 300 keV／μm and even smaller than unity for very high LET ions, which were far lower than those for cell killing. Therefore it is not simply dsb, but the non-repairable breaks that are associated with high biological effectiveness in the cell killing effect for high LET radiation. Here, we have examined the effectiveness of high LET neutrons on cell death in 19 mammalian cell lines including radiosensitive mutants.Fast neutrons were generated by the cyclotron at NIRS. The contribution of γ-rays was less than 5% of the total dose. The dose rate was approximately 0.7 Gy／min. Cells were irradiated through a 5 mm plastic plate to obtain full build up. X-rays of 200 kVp filtered with 0.5 mm Cu and 0.5 mm Al were used for reference. Some of the radiosensitive lines were deficient in DNA dsb repair such as LX830, M10, V3, SX9, SX10, and L5178Y-S cells. They showed lower values of RBE for fast neutron beams if compared with their parent cell lines, L5178Y, CHO-AA8, and SR-1. The average RBE value of dsb repair deficient cells was 1.23 ± 0.15 (mean ± standard deviation) in contrast to that of their parent cell group (normal group) of 1.86 ± 0.16. The other lines of human ataxia telangiectasia fibroblasts, irs 1, irs 2, irs 3 and irs1SF cells, which were also radiosensitive but known to be proficient in dsb repair (AT type group), showed a moderate RBE of　1.68 ± 0.15. The averages of these 3 groups were significantly different ($p < 0.05$) from each other, and the RBE value for dsb repair deficient cells was much lower than values for the other 2 groups ($p < 0.0005$). AT type cell lines are hypersensitive to X- rays, but behave like normal cells towards high LET neutrons. This suggests that the repair system which the AT type cell lines lack does not have a major role in causing the strong cell killing effects of neutrons. On the other hand, cell lines lacking dsb repair ability behave similarly towards X-rays and neutrons. Therefore dsb seems to be a key determining the cell killing effect of high LET radiations. We hypothesize that the main cause of cell death induced by high LET radiations is non-repairable dsb, which is produced at a higher rate than in low LET radiations.

[Publications]

Eguchi-Kasai, K., Murakami, M., Itsukaichi, H., Fukutsu, K., Kanai, T., Furusawa, Y., Sato, K., Ohara, H. and Yatagai, F.: Adv. in Space Res., 1

3．1　BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．3　Low Dose Irradiation-Induced Protective Function of Drug Metabolizing Enzyme System in Rat Liver Microsomes against Higher Doses of Radiation

Osami Yukawa and Tetsuo Nakajima

Keywords:low dose radiation, free radical, radical scavenger, drug metabolizing enzyme system, biological membrane.

As one of the mechanisms of adaptive responses induced by low dose irradiation, an increase in cellular free radical scavengers is postulated from the viewpoint that biological effects of low LET radiation are mainly due to free radicals generated following radiation. We have previously reported that whole body irradiation of male Wistar rats with 5cGy of X-rays caused an increase in liver cytosolic radical scavenging activity and a prior irradiation with 5cGy protected the subsequent 7.1Gy irradiation-induced decrease in microsomal drug metabolizing enzyme activity using hexobarbital as a substrate. Our present study dealt with changes in the components of the microsomal drug metabolizing enzyme system after 7.1Gy irradiation combined with 5cGy; we wanted to clarify the protective mechanism for this membrane-bound enzyme system by low dose irradiation against action of higher doses of radiation.Whole body irradiation of rats with 5cGy was carried out 1 day before irradiation with 7.1Gy and changes in drug metabolizing enzymes in liver microsomes were determined 5 days after the second irradiation. The microsomal drug metabolizing enzyme system is composed of NADPH-cytochrome P-450 reductase and cytochrome P-450 and the metabolizing activity is strongly dependent on microsomal phospholipids. Significant decreases in activities of NADPH oxidation and NADPH-cytochrome P-450 reductase, in the content of cytochrome P-450, and in the binding capacity of cytochrome P-450 for hexobarbital were observed after irradiation with 7.1Gy of X-rays alone. Irradiation with 5cGy prior to the 7.1Gy irradiation caused a marked protection of the decreases in activities of NADPH oxidation and of NADPH-cytochrome P-450 reductase and a complete protection of damages to the content and hexobarbital binding capacity of cytochrome P-450. Our previous study demonstrated that this enzyme system was inactivated by radiation-induced microsomal lipid peroxidation which was suppressed by liver cytosol. Therefore, the present study suggests that the substantial protection of the components of the drug metabolizing enzyme system, especially of cytochrome P-450 which is easily inactivated by lipid peroxidation, is due to the suppression of radiation-induced lipid peroxidation through the increase in radical scavenging activity following low dose irradiation. Analysis of inducible antioxidative substances present in microsomal membranes is in progress, in relation to the protection of membrane- bound enzymes.

3．1　BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．4　A Mechanism of Radiation-induced Activation of Protein Kinase C in Primary Cultured Rat Hepatocytes

Tetsuo Nakajima and Osami Yukawa

Keywords:rat hepatocytes, signal transduction , protein kinase C, lipid peroxidation, trolox

We have already demonstrated that active oxygens produced by radiation induce lipid peroxidation in primary cultured rat hepatocytes. In addition, we observed that protein kinase C (PKC) was activated following irradiation and that the activation was due to translocation of PKC molecules from cytosol to membranes. In the present experiment, we investigated the time course of the PKC activation and the relationship between PKC activation and lipid peroxidation after irradiation of cultured hepatocytes to elucidate further details of the activation mechanism and its cellular function.The time course of PKC activation was measured as an increase of PKC molecules in the membrane fraction of hepatocytes after irradiation with 50Gy of γ-rays. Phorbol -12,13- dibutyrate(PdBu) binding assay was used to determine the number of PKC molecules. PdBu binding activity in the membrane fraction began to increase 15 min after irradiation of the cells, reached a maximum 30 min after irradiation, and decreased 90 min after irradiation, indicating a rapid and transient activation of PKC following irradiation. The effect of trolox, a water soluble vitamin E derivative that has radical scavenging properties, was also investigated in hepatocytes to clarify the relationship between radiation-induced lipid peroxidation of hepatocytes and translocation of PKC. More than 85% of radiation-induced lipid peroxidation was suppressed by the addition of trolox(5-10mM) to the cells. Furthermore, trolox (10mM) treatment showed a simultaneous suppression of the PKC activation to the level observed in the non-irradiated controls without trolox. These results suggested that the translocation of PKC from the cytosol to the membrane is inhibited by the prevention of radiation- induced lipid peroxidation of the membrane.From these results, we concluded that the rapid and transient activation of PKC in hepatocytes is closely related to membrane lipid peroxidation induced by radiation. Further studies to determine PKC isoforms activated in the membrane and intracellular activators are in progress.

3．1　BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．5　Discontinuous Translation and mRNA Secondary Structure: Globin

Mitsuo Zama

Keywords: Discontinuous translation, mRNA secondary structure, globin

It is known for several proteins that growing polypeptide chains, which are being translated from mRNA, transiently accumulate as discrete size classes.This is due to discontinuities or pauses in the translation process, which may occur at specific sites in mRNA templates. In a series of studies for such proteins, silk fibroin, chicken type ■ collagen, colicin A and chloroplast photosystem ■ reaction center protein D1, we have so far presented evidence to support the view that the mRNA secondary structure of the protein■coding region is responsible for the discontinuous translation. Continuing from this now we have focused our attention on hemoglobin.It was shown in earlier studies by other authors that the rate of translation of hemoglobin mRNA
molecules from rabbit reticulocytes varies during the synthesis of the globin chains in a manner which suggests that slow steps are present during synthesis of specific regions of the globin molecules. We obtained the stability map of the local secondary structure of the mRNA for each of the rabbit reticulocyte α and β globin genes, by plotting the free energy of the optimal secondary structure of the local segment of the mRNA against the segment position along the base sequence of the mRNA. We found that the positions of the minima or lower free energy in the map for the α globin mRNA are approximately located at the regions where slow steps or pauses during synthesis of globin molecules are observed. This suggested that the synthesis pauses of globin chains arise from the mRNA secondary structure of the α globin gene. Clarification of the physiological significance of the discontinuous translation and its relation to the structure of the protein■coding region of mRNA might give us information on the origins and evolution of the nucleotide sequences of protein■coding genes.

[Publications]

Zama, M: Nucleic Acids Res. Symp. Ser., 35, 293-294, 1996.

3．1 BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．6 Formation of New Nitroxyl Radicals due to Chlorine Dioxide (ClO_2) Radical and Various Spin-Traps in Aqueous Solutions

Toshihiko Ozawa, Yuri Miura and Jun-ichi Ueda

Keywords:chlorine dioxide, ESR, spin trapping, Ti^{3+}, $KClO_3$, free radicals, oxidation

The reactivities of chlorine dioxide (ClO_2), which is a stable free radical, towards some water-soluble spin-traps were investigated in aqueous solutions by electron spin resonance (ESR) spectroscopy. The ClO_2 radical was generated from the redox reaction of Ti^{3+} with potassium chlorate ($KClO_3$) in aqueous solutions. When one of the spin-traps, 5,5-dimethyl-1-pyrroline N- oxide (DMPO), was included in the Ti^{3+}-$KClO_3$ reaction system, the ESR spectrum due to the ClO_2 radical completely disappeared and a new ESR spectrum [$a_N(1) = 0.72$ mT, $a_H(2) = 0.41$ mT], which differed from that of DMPO-ClO_2 adduct, was observed. The ESR parameters of this new ESR signal were identical to those of 5,5-dimethylpyrrolidone-(2)-oxyl-(1) (DMPOX) (Fig.12), suggesting the radical species giving the new ESR spectrum was assignable to DMPOX. A similar ESR spectrum consisting of a triplet [$a_N(1) = 0.69$ mT] was observed when the derivative of DMPO, 3,3,5,5-tetramethyl-1-pyrroline N-oxide (M 4 PO), was included in the Ti^{3+}-$KClO_3$ reaction system. This radical species was attributed to the oxidation product of M4PO, 3,3,5,5-tetramethylpyrrolidone-(2)-oxyl-(1) (M4POX) (Fig.1). When another nitrone spin-trap, α-(4-pyridyl-1-oxide)-N-t- butylnitrone (POBN) was used, the ESR signal intensity due to the ClO_2 radical decreased and a new ESR signal consisting of a triplet [$a_N(1) = 0.76$ mT] was observed. A similar ESR spectrum was observed when N-t-butyl-α-nitrone (PBN) was used as a spin-trap. This ESR parameter [$a_N(1) = 0.85$ mT] was identical to that of the oxidation product of PBN, PBNX (Fig.12). Thus, the new ESR signal observed from POBN could be assigned to the oxidation product of POBN, POBNX (Fig.12). These results suggested that the ClO_2 radical did not form stable spin adducts with nitrone spin-traps, but oxidized these spin-traps to give the corresponding nitroxyl radicals. On the other hand, the nitroso spin-traps, 5,5-dibromo-4-nitrosobenzenesulfonate (DBNBS), and 2-methyl-2-nitrosopropane (MNP), did not trap the ClO_2 radical. This result indicated that the unpaired electron of the ClO_2 radical was localized on an oxygen atom, because nitroso spin-traps cannot form a stable spin adduct with an oxygen-centered radical.

[Publications]

Ozawa, T., Miura, Y., and Ueda, J.: Free Radic. Med. Biol. 20, 837-841, 1996.

Fig.1 The structures of oxidized nitrone spin-traps:
(1) DMPOX (2) M4POX (3) PBNX(4) POBNX.

3．1 BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．7 Effects of Hydroxyl Radicals on the Ion Permeability of Planar Lipid Bilayers through Incorporated Purified Cardiac Ryanodine Receptor

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Keywords:ion channel, ryanodine receptor, planar lipid bilayer, hydroxyl radical

Ryanodine receptor (RyR), a Ca2＋-release channel, is involved in physiological Ca2＋-release from sarcoplasmic reticulum (SR) in both skeletal and cardiac muscles. Various oxidative stresses have been shown to alter the Ca2＋ permeability of the SR membranes. Reconstitution studies of the SR vesicles to planar bilayers have revealed that ionic current through the RyR channel is modified by oxidative stress [Stoyanovsky et al. (1994) Arch. Biochem. Biophys., 308, 214-221; Boraso and Williams (1994) Am. J. Physiol., 267, H1010-H1016]. In these reports, it is not clear if hydroxyl radicals are involved as the crucial oxidant and if the oxidative stress directly alters the ryanodine receptor molecule or indirectly affects the receptor through modification of lipids or accessory proteins which affect the RyR. In the present study, we have measured the effects of hydroxyl radicals on the ion permeability through purified cardiac RyR incorporated into the planar bilayer membrane.The effects of the hydroxyl radicals on the permeability of the bilayer without incorporation of the purified RyR were also measured. The cardiac RyR, purified from pig heart and reconstituted with asolectin as proteoliposome, was incorporated into a planar bilayer membrane. The RyR showed a single channel activity with a conductance of 731 pS in 900／200 mM (cis／trans) KCl and ion selectivity of PK:PCl＝1:0.03. These characteristics are similar to those reported for the RyR incorporated into planar bilayer membranes through SR vesicles. Hydroxyl radicals chemically generated by the reaction of H 2 O 2 and Cu(ethylenediamine) 2 at the cis compartment increased the open provability of the channel from Po＝0.27 to 0.94 after a lag time of about 2 min. On the other hand, no permeability change occurred in the lipid bilayer without incorporation of the purified RyR even at higher concentrations of hydroxyl radical generating system. The results indicate that the hydroxyl radicals directly modify the RyR channel protein and increase the Ca 2 ＋-release through it.Pretreatment of the RyR with SH reagent, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), quenched the reactivity of hydroxyl radicals with RyR. One or several SH groups, when blocked with DTNB, prevented hydroxyl radicals from enhancing the increase in ion permeability. These findings demonstrate that hydroxyl radicals react with some SH groups of the RyR channel protein and increase the Ca 2 ＋ release from SR through RyR, which may physiologically cause harmful effects on the cardiac muscle cells.

3．1．8 Expression of IL-1β mRNA in Mice after Whole Body X-Irradiation

Hiroshi Ishihara, Kumie Nemoto-Nojima, Izumi Tanaka, Gen Suzuki, Kazuko Tsuneoka, Kazuko Yoshida and Hiroshi Ohtsu

Keywords:interleukin-1β, radiation damage, myeloid cells, quantitative Northern hybridization, in situ hybridization

Ionizing radiation stimulates various kinds of biological reactions in mammalian cells. A gene for the interleukin(IL)-1β encoding radioprotector is induced by x-irradiation in normal mice cells. There are multiple profiles for increase in IL-1β mRNA after irradiation. Immediately after whole body irradiation at a high dose (20 Gy) of x-rays, monocytes／macrophages in spleen accumulate mRNA for IL-1β. The increased level of the message returns to the normal level within 120 min after the irradiation. This probably reflects an early stage of one of the signal transduction mechanisms triggered by x-ray-induced damages. In contrast, late response for induction of IL-1β gene is moderate and constitutive. When mice were irradiated with a sublethal dose (3-6 Gy) of x-rays, accumulation of IL-1β mRNA is observed several days after irradiation in spleen cells. Since the dose of 3Gy x- rays to C3H／He inbred mice induces myeloid leukemia at the highest incidence (20-30% of individuals), we analyzed the relationship between IL-1β expression and population change in spleen and bone marrow of irradiated mice.Normal mouse spleen has a number of cell types such as myeloid and lymphoid cells at various stages of differentiation. Spleens from 3 mice were isolated from whole body-irradiated C3H／He mice at 1, 3, 5, 7, 9, 11, 13, 15 and 17 days after irradiation. They were simultaneously analyzed by histochemical staining, flow cytometric analyses, CFU-GM assay and quantitative Northern blot hybridization. To avoid any effect of variation between mice groups, the similar experiments were done using three different lots of C3H／He mice. In the Northern analyses, the amount of IL-1β mRNA in the same amount of cells was measured. Increase in IL-1β mRNA was observed 5-9 days after irradiation. Fig.1A shows experimental results with the message peak 7 to 9 days after irradiation. Total cell number and CFU-GM amount decreased 1 day after irradiation and recovered to the normal level 15-17 days after irradiation. Between 5 to 9 days after irradiation, cell number in spleen partially recovered with an increase in contents of monocytes／macrophages. The time of the partial recovery was identical to that of the peak in IL-1β message level.

Differentiation of monocytes／macrophages results in increased expression of IL-1β mRNA. If monocytes ／macrophages are not differentiated, an increase in their content at 5-9 days leads to detection of accumulation of IL-1β mRNA in spleen by Northern analysis. We analyzed the heterologous spleen cells by in situ hybridization analysis (Fig.1B). Although monocytes／macrophages in spleen express a small amount of IL-1β mRNA before irradiation, a small number of them produce a large amount of the message.Results of the study imply that an increase in the relative ratio of monocytes／macrophages with differentiation is due to removal of lethally damaged cells in spleen and it is reflected as an increase in IL-1β mRNA level. Since IL-1β stimulates a wide

variety of myeloid and lymphoid cells to produce other cytokines, the finding probably reflects an early stage of recovering by hematopoietic cells after radiation damage.

[Publications]

Nemoto, K., Ishihara, H., Tanaka, I., Suzuki, G., Tsuneoka, K., Yoshida, K. and Ohtsu, H.: J. Radiat. Res., 36, 125-133, 1995.

Fig.1. (A) Each lane on the Northern blots contained 10μ■ of total spleen RNA from 3 mice sacrificed before (0 day) or on various days as indicated after ir (B) In situ hybridization probing IL-1β cRNA was performed using spleen cells before or after indicated days following irradiation. Each pair of microphotog

3．1　BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．9　Immediate-early, Transient Induction of the Interleukin-1β Gene in Mouse Spleen Macrophages by Ionizing Radiation

Hiroshi Ishihara, Izumi Tanaka, Kumie Nemoto-Nojima, Kazuko Tsuneoka, Cheeratana Cheeramakara＊, Kazuko Yoshida and Hiroshi Ohtsu (＊Visiting Scientist from Mahidol Univ.,Thailand)

Keywords:interleukin-1β, early response gene, transcriptional regulation, mRNA stability, in situ hybridization

Interleukin(IL)-1β is a multifunctional cytokine and has important roles in a variety of biological reactions. IL-1β is known as a radioprotector, since the survival rate for mice which have been irradiated rises after injection of the protein. We previously found that IL-1β gene is induced by x-irradiation in normal mouse spleen cells. The fact that the gene encoding radioprotector is responsive to exposure of ionizing radiation suggests the presence of radiation-responsive mechanisms to minimize damages by radiation. IL-1β mRNA level increases immediately after irradiation and decreases within 120 min in isolated spleen cells from normal mice. The immediate-early, transient induction reflects a signal transduction mechanism directed by the ionizing radiation. Therefore, we focused on the early regulation mechanisms, although IL-1β is also induced several hours after irradiation in a late response to radiation. Lymphoid and myeloid cells are found at various stages of differentiation in normal spleen. We determined the type of cells which causes the immediate-early induction of IL-1β gene after x-irradiation. Normal spleen cells were bound to one of the following primary antibodies; MOMA2 and F4／80 for monocytes／macrophages, anti-gra1 for granulocytes, anti-B2／20 for B lymphocytes, anti-CD3 for T lymphocytes. After mixing them with the secondary antibodies which were bound to DynabeadsTM, different types of cells were successfully separated using a magnet. After removal of the antibodies by preincubation, the cells were x-irradiated at 20 Gy. The irradiated cells were fixed on a slide glass and analyzed by in situ hybridization probed with 35S-labeled antisence IL-1β RNA. As shown in Fig. A, an increased level of mRNA for IL-1β was observed after irradiation in the cells which bound to MOMA2 and F4／80. In contrast, cells which had gra-1, B2／20 or CD3 did not express IL-1β messages before and after irradiation. The results show that monocytes／macrophages in the normal spleen express IL-1β gene immediately after x-irradiation. To the further molecular studies, we used a macrophage cell line, L-8704, derived from C3H／He mice. An increase in mRNA for IL-1β was observed immediately after x-irradiation, similar with normal spleen cells. When the cells were irradiated in the presence of protein kinase inhibitors, x-ray responsive induction of IL-1β disappeared. This shows that x-ray-responsive immediate expression of IL-1β is controlled by signal transduction mechanisms including protein kinase networks at the transcriptional level. To confirm the contribution of transcriptional regulation, the nuclear run-on assay was performed. Isolated nuclei from non-irradiated and irradiated L-8704 cells were incubated with α-32P-UTP to label transcripts. The radioactive RNAs were hybridized with IL-1β cDNA which binds nitrocellulose. As shown in Fig. B, IL-1β-related transcripts were synthesized when nuclei from irradiated cells were used. This shows that the

immediate-early induction of IL-1β is regulated at the transcriptional level. As described above, x-ray-responsive immediate-early induction of gene for IL- 1β which encodes a radioprotector is transcriptionally regulated in monocytes／macrophages. This probably reflects the fact that x-irradiation stimulates radioprotective mechanisms, including IL-1β expression, via signal transduction. To clarify the mechanisms, further analysis of the events from irradiation to initiating transcription is necessary.

[Publications]

Ishihara, H., Tanaka, I., Nemoto, K., Tsuneoka, K., Cheeramakara, C., Yoshida, K. and Ohtsu, H.: J. Radiat. Res., 36, 112-124, 1995.

Fig.1. (A) Spleen cells were separated using the indicated surface antibodies. Before and immediately after irradiation of 20 Gy x-rays, they were analyzed

3．1 BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．10 Molecular Dynamics (MD) Simulation on Normal and Damaged DNA

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Keywords:molecular dynamics, DNA, thymine dimer, repair enzyme, T4 endonucleaseV (T4 endo V)

Conformational change in damaged DNA is a signal for repair enzymes to detect and restore the damaged site. This study is aimed at clarifying this process at the molecular level by applying MD. We have taken as an interesting example, cyclobutane-type pyrimidine dimer as the damaged site and its repair enzyme, T4 endonuclease V (T4 endo V). Both molecular structures are already known and related reaction mechanisms have been discussed in terms of their crystallographical structures. We investigate whether those mechanisms hold in dynamic process in solution at room temperature. Our MD simulations have been divided into three parts, MD of DNAs, MD of T4 endo V, and MD of complexes of DNAs and T4 endoV. The first of these is discussed here. We considered two normal DNAs, DNA1: d(GCGGATGGCG)・d(CGCCTACCGC), DNA2: d(GCGGTTGGCG)・d(CGCCAACCGC) and a damaged DNA, DNA3: d(GCGGT＾TGGCG)・d(CGCCAACCGC), where T＾T is the cyclobutane-type thymine dimer. These DNAs are important, because repair mechanisms have been crystallographically discussed on these decamer DNAs. The DNAs are put in the center of boxes with waters, DNA1: 49.8 x 38.4 x 39.1 A 3, 2400 waters, DNA2: 52.2 x41.6 x 41.6 A 3 , 2741 waters, and DNA3: 52.3 x 41.5 x 41.8 A 3, 2750 waters. AMBER ver 4.0 force field is assumed. Continuous MDs are run with a constant volume having a periodic boundary condition and constant temperature of 300K toward thermodynamic equilibrium. Fig.15 shows their conformations at a transient stabilized time region around 140 ps. A slight difference between DNA1 and DNA2 suggests that local conformation of DNA is slightly dependent on sequence of bases. The damaged DNA, DNA3, shows a specific conformational change "bend" around the thymine dimer. This conformational change is considered to be a signal to the repair enzyme when both types of molecules come closer, after the enzyme recognizes the damage site through hydration layers of both sides, i.e. the DNA and the enzyme. This means the distribution of waters around damaged DNA is also important as a signal to be detected when both molecules contact at a distance within each hydration layer. Study of this hydration structure and analysis methods of conformations is continuing. Before we study the MD of the enzyme alone and complexes of DNA and the enzyme, examination and verification of MD itself, including evaluation of the force fields, are still needed to output more reliable results.

[Publications]

Pinak, M., Yamaguchi, H. and Osman, R.: J. Radiat. Res., 37, 20-28, 1996.

Fig.1 Conformations of normal DNA1 and DNA2, and damaged DNA 3, at a transient stabilized time around 140 ps.

3．1　BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．11　Radiolytic Products of Bromodeoxyuridine in Solids Produced by Co-60 Gamma-Rays and Monoenergetic Soft X-Rays at the K-Absorption Edge of Bromine

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Keywords:bromodeoxyuridine, DNA damage

In order to investigate DNA damage due to Auger cascades in bromodeoxyuridine (BrdU), BrdU mixed with other nucleosides, as a model of DNA, was irradiated in solids by gamma-rays, and monoenergetic X-rays at around the K-absorption edge of bromine (13.47 keV). The main products of BrdU were deoxyuridine produced through debromination, and bromouracil produced through the decomposition of sugar group. The rates of the debromination and the nucleobase release of additives were markedly increased in the mixed sample. This observation indicated that the additives surrounding BrdU efficiently supplied protons and then decomposed. The major products by X-rays were the same as those by gamma-rays, indicating that the Auger cascade in bromine atoms did not produce specific products. The production rates for all products from the mixed sample were about 2.5- times higher at 13.51 keV (above the K-absorption edge) than at 13.43 keV X-rays.

3．1　BIO-MEDICAL SCIENCE Biochemistry and Biophysics

3．1．12　Dependence of RBE on LET and Particle Species

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Keywords: Heavy ions, mammalian cells

To clarify the difference in RBE by different heavy ions with the same LET beams, we exposed mammalian cells (V79 and HSG) to accelerated heavy-ion beams ([3]He, 12C, and 20Ne), and the cell-killing efficiencies were measured. The survival curves of V79 cells for lower LET beams had large shoulders (3.8 Gy as Dq, for both [3]He- and 12C-ions, at 20 keV／μm), and those of HSG cells had small shoulders (1.2 and 1.8 Gy for [3]He- and 12C-ions, respectively, at 20 keV／μm). The shoulders were reduced by an increment of the LET. The D10 values, for all cases, decreased with an increment of LET to a minimum at about 100-300 keV／μm, and then increased in a much higher LET region. The LET-D10 curves of the V79 cells for [3]He-, 12C-, and 20Ne-ions were spreader. In the middle (30-90 keV／μm) LET region, the lowest D10 at the same LET radiation was found by the [3]He-ion, and the values were intermediate for the 12C-ions and highest for the 20Ne-ions. Analogous figures were found for HSG cells. The D10 curves produced by the [3]He-ions were clearly lower than those produced by the other ions.

3．2 BIO-MEDICAL SCIENCE Cell Biology

3．2．1 A Major LINE of Bombyx mori , L1Bm

Sachiko Ichimura, Kazuei Mita and Kimihiko Sugaya

Keywords:retrotransposon, LINE, Bombyx

。。Radiation effects on genetic stability would depend on features of transposons. Most eukaryotic genomes contain retrotransposons, mobile genetic elements that amplify via an RNA intermediate. Like retroviruses, these elements encode gag and reverse transcriptase (RT) genes. Long terminal repeats (LTRs), however, are not commonly present, and the elements fall into two groups, LTR and non-LTR retrotransposons. Non-LTR retrotransposons have also been called LINE-like elements because long interspersed elements (LINEs) of mammals possess a typical structure of non-LTR retrotransposons. Some LINEs representing human L1 family, characterized by frequent truncation at 5' end and the resultant short 3' side sequences resemble short interspersed elements (SINEs) in that the elements have 3' tails of poly A traces or a simple A-rich repeat. In fact, early reports of an avian LINE, CR1, had predicted it to be a SINE, in spite of the absence of the poly A tail. 。。We observed that the intron of fibroin gene contains a highly abundant interspersed repetitive unit (Ichimura, S. and Mita, K.: J. Mol. Evol., 35, 123, 1992). We had sequenced a Bombyx mori genomic clone containing the unit and found a SINE-like repetitive sequence. Further sequencing of the genomic clones, however, showed that the element is a major non-LTR retrotransposon of B. .mori and evolutionally classified in the same group of LINE-like elements of Drosophila and Mosquito . The element, L1Bm, has a molecular size of 5.3kb containing gag and RT sequences with an A-rich 3'-tail. Most elements are truncated at the 5'-side and the abundant incomplete 3'-side sequences are dispersed in the genome. In the genome of B. mori some LINE-like elements, R1Bm, R2Bm, DONG and TRAS1 have been discovered. These LINE-like elements, however, are not so frequent and are liable to favor specific genomic environments. BMC1 reported as an abundant dispersed LINE-like element was a partial sequence of L1Bm and therefore, in insect L1Bm2 is the first complete unit corresponding to a major LINE in B. mori. Interestingly new LINEs have been identified recently in non-mammalian organisms, chicken, lilium speciosum and trypanosoma cruzi.

3．2 BIO-MEDICAL SCIENCE Cell Biology

3．2．2 Complement C1s, a Classical Enzyme with Novel Functions at Endochondral Ossification Center: Immunohistochemical Staining of Activated Cls with a Neoantigen Specific Antibody.

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Keywords : neoantigen specific antibody,complement Cls,cartilage, hypertophic chondrocytes, programed cell death, ultrastructure

。。In the classical complement cascade, Cls activated by Clr cleaves C2 and C4 which are known as Cls sole substrates. Recently, however, we showed that Cls degrades type「」collagen and gelatin and activates zymogen of matrix metalloproteinase-9. Type「」collagen is a major constituent of cartilage matrix and MMP-9 is synthesized by chondrocytes. MMP-9 is colocalized with Cls at the ossification center. Therefore, Cls is thought to have a role in cartilage degradation. 。。Cartilage consists of chondrocytes and extracellular matrix which is composed of several types of collagen and various kinds of proteoglycans. For long bone development and growth, chondrocytes pass through resting, proliferating, maturing, hypertrophic and degenerating stages. This naturally occurring cell death aids in tissue remodeling. Degenerated chondrocytes disappear leaving enlarged lacunas and surrounding matrix which is eventually degraded and the site is replaced by bone marrow. Programmed cell death of chondrocytes is accompanied by an expression of an s-myc protein and tissue transglutaminase. In most tissues dead cells are usually cleaned away by phagocytotic cells. In cartilage, an avascular tissue, however, degenerating cells disappear before monocyte-like phagocytotic cell invasion. Degrading enzymes may play a major role in the cleaning mechanisms in cartilage. We have shown that Cls synthesis increases in accordance with chondrocyte differentiation both in vivo and in vitro. Cls synthesized by chondrocytes or delivered by blood stream, may participate in chondrocytes and matrix degradation. Cls has to be activated in situ to achieve this function. In order to prove this possibility by an immunohistochemical technique,we designed inactive and active Cls spacific antibodies, RK4 and RK5, respectively. On activation, hamster Cls is cleaved between 423Arg and 424Ile generating a new NH［イ］-terminal site of the L chain. RK5 recognizes this new epitope of the L chain and RK4 recognizes an uncleaved peptide around the cleavage site. 。。Cls secreted from primary hamster embryo fibroblasts was 100。 inactive form.

On the other hand, Cls secreted from malignant hamster embryo fibroblasts was 100。 activated. RK3, an antipeptide antibody recognizing around the active center, reacted with both active and inactive Cls. RK4 reacted only with inactive Cls. By contrast, RK5 recognized the L-chain of activated Cls but not the inactive one. Cls activation was expected to be visualized in situ using these antibodies. RK4 reacted strongly with cytoplasm of hypertrophic chondrocytes. In contrast, RK5 stained Cls in cartilage matrix around the invading vessels. In addition, we found that Cls degraded decorin which is one of the proteoglycans present in cartilage. These results strongly suggest participation of Cls in cartilage remodeling.

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3．2 BIO-MEDICAL SCIENCE Cell Biology

3．2．3 Comparison of the 5' Upstream Region of the Evolutionarily Equivalent Polyubiquitin Gene of Humans and Chinese Hamsters

Mitsuru Nenoi, Kazuei Mita, Sachiko Ichimura and Iain L. Cartwright。 (。Univ. of Cincinnati, U.S.A.)

Keywords:polyubiquitin gene, evolutionarily equivalent

Ubiquitin is a highly conserved small protein involved in many cellular processes. Ubiquitin is thought to function in the UV- induced transcriptional induction response (UV response) by participating in the metabolism of the required transcription factors AP-1 and NF-ｶﾊB. 。。We previously showed that the expression of the polyubiquitin gene UbC of HeLa cells, which encodes nine tandemly repeated ubiquitins with no intervening spacer, is enhanced after UV irradiation and TPA treatment. Further, we showed that the Chinese hamster polyubiquitin gene CHUB2, encoding eight tandemly repeated ubiquitins followed by a non-ubiquitin polypeptide of 50 a.a., is an evolutionary equivalent of the human UbC gene on the basis of the sequence homology in the 5' and 3' untranslated regions. However, we observed that the expression of the CHUB2 gene is not apparently inducible by UV light in V79 Chinese hamster lung fibroblasts. No induction of the ubiquitin genes by UV light or TPA has been reported in Chinese hamster ovary cells (CHO) either. 。。In order to initiate investigation of the mechanisms behind the dissimilar regulation of the Chinese hamster CHUB2 gene and the human UbC gene, we determined the nucleotide sequence up to -4345 bp from the transcriptional initiation site of the CHUB2 gene (deposited in the DDBJ, EMBL and GenBank nucleotide sequence databases under the accession number D63782), and compared it with that of the human UbC gene. As shown in Fig.1, the 5' control region of the CHUB2 gene is not characterized by clustered recognition sequences for transcription factors as seen in the UbC gene. There are no AP-1 sites, few NF-ｶﾊB sites and even fewer SP-1 sites, reflecting the low GC content of this region compared with the corresponding region of the human UbC gene. It is considered likely, from this result, that the absence of AP-1 sites in the less organized 5' control region of the CHUB2 gene may well be responsible in part for the dissimilar regulation of the two genes by UV light and TPA, despite the fact that these genes are evolutionarily equivalent.

[Publications]

Nenoi, M., et al.: Gene, 179, 297-299, 1996.

Fig.1 Schematic representation of overall structure of the UbC and CHUB2 genes. Sites of a perfect match to the consensus sequences for transcription factors

3．2 BIO-MEDICAL SCIENCE Cell Biology

3．2．4 Heterogeneous Structure of the Polyubiquitin Gene UbC of HeLa S3 Cells

Mitsuru Nenoi, Kazuei Mita, Sachiko Ichimura, Iain L. Cartwright[ア], Ei-ichi Takahashi[イ], Masatake Yamauchi and Hideo Tsuji ([ア]Univ. of Cincinnati, U.S.A.; [イ]Otsuka Pharmaceutical Co.)

Keywords:variable number of tandem repeat, unequal crossover, polyubiquitin gene

。。The nucleotide sequence of the polyubiquitin gene UbC of HeLa S3 cells and its upstream region were determined and characterized. Recognition sequences for the transcription factors HSF, NFｶﾊB, AP-1(c-jun), NF-IL6 and Sp1 were found in the upstream control region, a result consistent with the observation of a distinct regulatory response for the UbC gene compared with that of another polyubiquitin gene UbB. Employing a PCR procedure to amplify the entire coding region from genomic DNA, we found a heterogeneity in the repeat number (eight and nine repeats) of the ubiquitin coding units, which resulted from an apparent deletion of either the seventh or the eighth unit in the predominant nine-ubiquitin-unit coding gene. In addition, by comparison with the nucleotide sequence of the UbC gene of human leukocytes previously determined, we found a significant number of nucleotide discrepancies. However these discrepancies could be substantially reduced by realigning the units so that the first and second ubiquitin units of the sequence determined here are translocated to the boundary between the eighth and the ninth units.

[Publications]

Nenoi, M., et al.: Gene, 175, 179-185, 1996.

3．2 BIO-MEDICAL SCIENCE Cell Biology

3．2．5 Distribution of Casein-like Proteins in Various Organs of Rat

Makoto Onoda and Hiroshi Inano

Keywords:casein, mammary gland, immunohistochemistry

。。The ultimate function of the mammary gland is to produce milk which contains important substances for newborns, and it is known that the growth and differentiation of the mammary gland are basically controlled by multiple interactions of several peptide and steroid hormones from endocrine organs, such as the pituitary and ovary. Casein is one of the major components of milk which is produced and secreted by the mammary gland epithelium in a hormonally controlled manner during the lactational period. Indeed, appreciable amounts of casein mRNAs are present in the rat mammary gland during pregnancy and lactation. Subsequently, casein as well as ヲチ-lactalbumin represent specific molecular markers of the secretory activity, and the degree of differentiation of epithelial cells in the mammary glands. 。。On the other hand, the determination of casein by radioimmunoassay in the serum of patients with various malignancies, not only of the breast but also of other organs, raised concern about the specificity of casein production as a marker of the mammary gland origin. In this context, we have attempted to clarify the presence of caseins and casein-like proteins in a variety of organs, since caseins could be a useful molecular marker to understand the secretory function and the differentiation mechanisms of epithelial cells not only in the mammary gland but in many other organs. 。。Casein-like proteins were detected in various organs of rat by using a specific antiserum raised against rat milk caseins. The antiserum specifically recognized ヲチ｣ｱ-, ヲチ｣ｲ-, ヲﾂ- and ヲﾃ-caseins in the rat milk by Western blot analysis. Immunohistochemical studies of this antiserum on the formalin fixed mammary glands showed that immunoreactive caseins localized on the apical portion of cytoplasms of lactating mammary epithelial cells and in the secretion of lumen, which indicates a directional secretion of caseins to the lumen from the mammary epithelial cells. Immunoreactive substances using this antiserum were detected in various organs, including the pancreatic ducts and islets of Langerhans, the secretory ducts of salivary glands, zona fasciculata cells and ganglion cells of the adrenal gland, distal tubes and convoluted collecting tubes of the kidney, epithelial cells of bronchioles and large pneumocytes of the lung, hair follicles, sebaceous glands and the pickle cell layer of the skin, uterine glands and epithelium of the endometrium, hepatic bile ducts and the brain. In Western blot analysis, major immunoreactive substances in the above organ extracts showed a similarity in molecular weight to ヲチ｣ｲ-casein of rat milk. Skin was the only tissue which expressed both ヲチ｣ｲ- and ヲﾂ-caseins. These findings suggest that the ヲチ｣ｲ-casein-like substance is not only localized in the mammary gland but also in a variety of organs and may play an important role as a functional molecule in those organs. 。。One noteworthy finding was that the casein-like substances localized in the epithelium of exocrine ducts in a variety of organs, such as pancreas, salivary gland, kidney and liver. In addition, the hormonally controlled organs including the adrenal gland and uterus also expressed the casein-like substances. Another observation in this study was that the ヲチ｣ｲ-casein-like protein was a major component in the casein-like protein expressed by organs. In our understanding, this

is the first time that the ヲチ」ｲ-casein molecular species was identified in various organs by Western blot analysis with a specific antiserum. One possible explanation for this phenomenon is the hormonal regulation of casein expression in a variety of organs. It is well understood that the syntheses of the caseins and their mRNAs are dependent upon hormonal control. A significant level of mRNAs for caseins is found in virgin rat mammary glands in which ヲチ-casein mRNA is present more than ヲツ- and ヲテ-casein mRNAs. The concentration of mRNA for ヲツ- casein becomes higher than that of other casein mRNAs after gestation. Therefore, in our study, it is likely that the ヲテ-casein-like protein was not detected by immunoblot analysis in the various organ extracts obtained from non-pregnant rats, and that most of these organs were abundant in the ヲチ-casein-like protein (especially, ヲチ」ｲ-casein-like protein) except the salivary gland. These observations may reflect the hormonal regulation of casein-like proteins in various organs as well as in the mammary gland. ｡｡ Although the localization of caseins in a variety of organs is now certain, the physiological roles of casein and casein-like proteins remains obscure. There are interesting reports which imply some possible roles of caseins. Cytotoxic T lymphocytes (CTL) expressed members of the casein gene family, such as ヲチ-, ヲツ- and ヲﾊ-caseins. Casein micelles act as a vehicle by which perforin, an important cytolytic mediator released from CTL upon antigenic stimulation, is delivered onto the surface of target cells.

Furthermore, low levels of mRNA for ヲチ-casein was detected in thymus by Northern blot analysis after PCR amplification. This is consistent with our finding that thymus extract contained ヲチ」ｲ-casein-like substance. Given these observations, caseins may be important as a sort of carrier protein in thymus, and CTL function. ｡｡Although the specific anti-rat casein antiserum we generated provides a useful tool to analyze the mode of expression of the respective genes by not only immunnohistochemistry but by Western blotting, cloned cDNA probes in general would be superior to immunological methods for the detection of specific markers in various organs, therefore, the recognition of mRNAs for caseins or casein-like substances by specific cDNA probes would be better approach to clarify the presence and localization of casein-like proteins in various organs. Furthermore, certain specific cDNAs will provide better understanding with regard to the regulation mechanisms of respective genes by steroid and peptide hormones in various organs.

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3．2 BIO-MEDICAL SCIENCE Cell Biology

3．2．6 Cloning and Sequencing for the Largest Subunit of Chinese Hamster RNA Polymerase「」 Gene

Kimihiko Sugaya, Shun-ichi Sasanuma,Junko Nohata, Terumi Kimura, Hideo Tsuji, and Kazuei Mita

Keywords: gene structure, RpII, RT-PCR, SCE, mutation site

。。Twenty-five temperature-sensitive mutants were isolated from Chinese hamster CHO-K1 cells after mutanization with N-methyl- N'-nitro-N-nitrosoguanidine. One of the mutants, tsTM4, exhibited abnormal induction of sister chromatid exchanges (SCEs) along with the decrease of DNA synthesis in the cells arrested in S phase at the non-permissive temperature. The cloning of the human gene complementing the defect of tsTM4 was performed. Then, we have isolated the gene for the human RNA polymerase「」 largest subunit (Rp「」 LS) and determined its genomic organization. The genomic fragments containing the whole coding regions of the human Rp「」 LS complemented the genetic defect of tsTM4. The Northern analysis showed that both Rp「」 LS genes (hamster and human) were transcribed at the non-permissive temperature in the transformant. These facts strongly suggest that a genetic defect in the Rp「」 LS gene would be responsible for the abnormal induction of SCEs in the tsTM4. To search for mutations in the Rp「」 LS gene, we have analyzed the whole region of the Rp「」 LS cDNAs derived from tsTM4 and CHO-K1 cells, and identified the mutation site by determining the sequences completely (Fig.1).

Fig.1. Cloning of Chinese hamster RNA polymerase「」 largest subunit. The black boxes (I-「」) in the schematic diagram of the Rp「」 LS gene product represent the regions conserved between the largest subunit of eukaryotes and ｦｯ' subunit of E. coli RNA polymerse. The hatched box (CTD) represents the carboxy-terminal heptapeptide repeat domain. The mutation site for tsTM4 is indicated by an asterisk. Location of cDNA clones are indicated above the schematic diagram. Sequenced regions are shown as horizontal lines with vertical bars as determined terminuses. The PCR primers to clone the whole coding region of the mutant Rp

「」 LS are indicated below the schematic diagram with arrow heads for direction. 。。

3．3 BIO-MEDICAL SCIENCE Immunology and Hamatology

3．3．1　E?ects of Fractionated Irradiation on Murine Megakaryocyte Progenitor Cells

Kaoru Tanaka and Eiichi Kojima

Keywords:megakaryocyte progenitor cells (CFU-Meg), fractionated irradiation, mice

Hematopoietic systems are the most sensitive systems in the body to radiation. Only a few studies have attempted to examine the effects of fractionated irradiation on hematopoietic progenitor cells in mice. We investigated the effects of in vitro fractionated irradiation with two equal split-doses on megakaryocyte progenitor cells (CFU-Meg) in the bone marrow and spleen of mice．。。Female BALB。ソc mice, 10-20 weeks old, were used for this study. CFU-Meg cultures were prepared according to the fibrin clot culture system of Kuriya et al. Femoral bone marrow (5。゜10」オcells。ソml) or spleen cells (50。゜10」オcells。ソml) were suspended in culture medium containing 10。◆ spleen lymphocyte-conditioned medium (as a source of colony-stimulating factor) and fibrinogen solution and then were plated. The fibrin clots were first exposed to X-rays (200kVp, 20mA, 0.7Gy。ソmin) at graded doses (0.1-1.9Gy) and were kept at 37。◆ for various times (1, 2, 3, 4, 5, and 24h). Then, they were irradiated a second time at the same doses as the first ones. Four days after irradiation and incubation, the cultures were fixed with 5。◆ glutaraldehyde and were stained for acetylcholinest-erase activity. Colonies consisting of 4 or more acetylcholinesterase-positive cells were scored as CFU-Meg. 。。The results were evaluated by comparison of the dose-survival relationships of CFU-Meg irradiated with a single dose and split doses of X-rays. The radiation-dose response curves for CFU-Meg in both bone marrow and spleen after a single irradiation with X-rays exhibited no shoulder with a single slope. A similar profile was found in the dose response curves after split doses of X-irradiation. The Do values of the dose response curves were measured at various time intervals between two equal split doses of X-irradiation. The data indicated that there was an apparent absence of recovery in the survival of femoral CFU-Meg after the fractionated doses of irradiation (Fig.1) and the small recovery of fractionated irradiation was found at 3 and 24h intervals for splenic CFU-Meg. In relation to the concentration of cultured cells, the dose response curves for CFU-Meg in the bone marrow slowed with the increase of the plated cells. The present studies also showed that the slope of the spleen dose-survival curves was slow in contrast with that of bone mallow, but the concentration of cultured spleen cells was usually ten times that of cultured bone marrow cells. When the sizes of individual colonies were examined by counting the number of acetylcholinesterase-positive cells per colony, there was little difference in the distribution of colony sizes between groups having single and fractionated doses of irradiation.

Fig.1 Changes in the Do values at different time intervals for single and two equal split doses of irradiation.

3．3 BIO-MEDICAL SCIENCE Immunology and Hamatology

3．3．2　Pre-T Cell Activity in the Bone Marrow during Early Stages after

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Keywords :pre T cell, strain difference, radiation-induced T cell lymphomas

。。It is known that exposure of B10. Thy 1.1 mice to fractionated X-irradiation induces a high incidence (。 ◆95。◆) of thymic lymphomas, whereas the incidence for STS。ｿA mice is very low (。◆8-9。◆). 。。Under the influence of an irradiated host environment, radiation-induced thymic lymphomas can originate from cells present in nonirradiated thymus grafts (Muto et al. Cancer Res., 43, 3822, 1983). Injection of the syngenic normal bone marrow cells after irradiation has been shown to enhance the progressive regeneration of the radiation-injured thymus and effectively prevent the development of thymic lymphomas. Thus, the cellular processes leading to the development of thymic prelymphoma cells seem to have involved a complex process in which the interactions between target cells for neoplastic transformation and thymic microenvironment and also bone marrow were of critical importance. 。。To investigate the cellular mechanism of strain difference with regard to radiation-induced lymphomagenesis, we established the STSA.Thy 1.1 strain by introducing Thy 1.1 marker from the B10.Thy 1.1 strain into STS。ｿA strain by 12 repeated backcrosses followed by brother-sister matings. 。。Using B10.Thy 1 congenic mice and STS。ｿA.Thy 1 congenic mice, we first investigated the radiosensitivity of pre-T cells in the bone marrow from B10.Thy 1.1 and STS。ｿA. Thy 1.1 mice. Bone marrow cells were taken from individual B10.Thy 1.1 and STS。ｿA.Thy 1.1 mice at various times after leukemogenic irradiation and a limited number of these cells (6 X 10ｺｳ-1 X 10ｺｵ) was injected intrathymically into 3.78 Gy-irradiated B10.Thy 1.2 and STS。ｿA.Thy 1.2 mice, respectively. One month after transplantation, the thymocytes were stained with FITC-labeled anti-Thy 1.1 and biotin-labeled anti-Thy 1.2 followed by avidin-PE, and the percentage of donor-type (Thy 1.1。ﾜ) cells was analyzed. The titration curves were drawn by limiting dilution analysis according to a Poisson distribution, and the number of pre-T cells in the bone marrow from B10.Thy 1.1 and STS。ｿA.Thy 1.1 mice after irradiation were evaluated. The results indicated that thenumber of pre-T cells in the bone marrow from B10.Thy 1.1 mice was severely depressed during the time up to 70 days after irradiation, while that from STS。ｿA.Thy 1.1 mice recovered to 22.4。◆ of that of non-irradiationed mice. 。。Moreover, leukemogenic irradiation resulted in a sustained depression of the level of mixed lymphocyte reactivity (MLR) of the thymus from B10.Thy 1.1 mice; in contrast, MLR of the thymus from STS。ｿA mice after irradiation was higher than that from the control group. TL-2 positive cells which were not expressed on normal thymocytes of B10.Thy 1.1 mice appeared significantly in the thymocytes of irradiated B10. Thy 1.1 mice, but the proportion of TL-2 positive thymocytes from irradiated STS。ｿA mice did not change when compared with normal thymocytes of STS。ｿA mice. Abnormal expressions of IL-2Ron thymocytes of irradiatedB10. Thy 1.1 mice were observed, but not on those of irradiated STS。ｿA mice. 。。Thus, in the B10. Thy 1.1 mice, irradiation (1.61 Gy X 4) causes atrophy of the thymus as well as depletion of pre T cells in the bone marrow. Under these conditions, a differentiation arrest and。ｿor abnormal T cell differentiation might occur among regenerating immature lymphoid cells, and some of

these regenerating cells may then undergo preneoplastic change or transformation due to induced abnormal gene expression followed by genomic instability and chromosome aberrations. On the other hand, radioresistant T cell precursors from STS。ﾘA mice appear to undergo normal differentiation. This might lead to prevention of the appearance of prelymphoma cells in irradiated STS。ﾘA mice.

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3．3 BIO-MEDICAL SCIENCE Immunology and Hamatology

3．3．3 Possible Induction of GVL E?ect against a Leukemia Refractory to Anti- leukemia Response in Usual MHC-compatible, Allogeneic Bone Marrow Transplantation

Shiro Aizawa, Hitoko Kamisaku and Toshihiko Sado

Keywords:CD8。ワ, T cells, GVL effect, GVHD, leukemia

。。Our previous results for a murine model indicated that GVL effect against leukemia LE750 could not be induced in MHC-compatible allogeneic BMT of leukemia-bearing C3H mice, whereas a significant GVL effect was observed against leukemia 8313. Furthermore, we showed that CD8。ワ T cells contaminating donor BM were consistently critical for the induction of GVL effect in allogeneic BMT of leukemia-bearing C3H mice and, furthermore, CD8。ワ T cells of MHC-compatible, allogeneic AKR donor mice could preferentially induce the GVL effect without significant lethal GVHD. 。。To elucidate cellular factors which influence sensitivity of leukemias to anti-leukemic response induced in allogeneic BMT of leukemia-bearing host, we examined why a significant GVL effect can not be induced against a leukemia (LE750) in ordinary MHC-compatible, allogeneic BMT. The resistance of LE750 leukemic cells to the induction of GVL effect was attributed to neither less sensitivity to lysis by minor H antigen-specific, cytotoxic T cells nor an immuno-suppressive activity of LE750 leukemic cells in leukemia-bearing mice. To investigate the significance of dose effect of cells responsible for the induction of GVL effect, we used CD8。ワ T cells of AKR donor mice which have been shown to preferentially induce GVL effect without lethal GVHD and therefore allowed us to increase the cell number of CD8。ワ T cells in the donor bone marrow inoculum. Although the addition of unprimed CD8。ワ T cells into the donor inoculum could not induce a significant GVL effect against LE750 leukemia, CD8。ワ T cells of donor mice which were primed with host cells manifested improvement in survival and the effect of the inoculation of primed CD8。ワ T cells was dose-dependent. The results indicate that the outcome of anti-leukemic response in allogeneic BMT of leukemic recipients may be determined, at least in part, by the balance between the malignancy of leukemias and the number of anti-leukemic effector cells.

[Publications]

3．3 BIO-MEDICAL SCIENCE Immunology and Hamatology

3．3．4 of Fractionated Radiation Exposure on Hematopoietic Potentials in Radiation-Induced Thymic Lymphoma-Susceptible B10 Mice and - Refractory C3H Mice

Hitoko Kamisaku, Shiro Aizawa, Kumie Nemoto, Masanobu Kitagawa。?, Yoshinori Ikarashi, and Toshihiko Sado (。口okyo Medical and Dental University, Tokyo)

Keywords:fractionated radiation, hematopoiesis, thymic lymphoma

We previously reported from dose-response analysis of thymocyte precursors by intrathymic injection that the number of thymocyte precursors present in the bone marrow of fractionated whole-body X-irradiation (FX) -treated B10 mice was greatly reduced as compared to that present in the normal bone marrow. The results suggested that the depletion of pre T cells in the bone marrow following FX-treatment was an important cellular events that led to the development of thymic lymphomas. 。。In order to explore the signi?cance of the shortage in supply of pre T cells from the bone marrow during FX-induced thymic lymphomagenesis, thymocyte precursor potentials in bone marrow of FX-treated B10 mice was compared to that of lymphomagenesis-refractory C3H mice. Furthermore, the recovery of other hematopoietic activities following FX-treatment was also examined. The results showed that the frequency of pre T cells in the bone marrow of FX-treated mice was greatly reduced to a few percent 4 weeks after irradiation in not only lymphoma-prone B10 but also -refractory C3H mice. On the other hand, the activities of hematopoietic stem cells (CFU-s and GM CFU-c) in the bone marrow of FX-treated mice were reduced to a few percent of those in normal bone marrow 3 days after irradiation but progressively recovered to about 20 to 50 。? 4 weeks later . The kinetics for the numbers of pre B , mature T and B cells in FX-treated mice also showed that the regeneration of all lymphoid lineage cells was severely delayed in both strains of mice as compared to that of Gr-1。ℑ polymorphonuclear cells. These results indicate that fractionated irradiation severely injured overall lymphopoiesis more than hematopoiesis including CFU-s, GM CFU-c and polymorphonuclear cells.

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3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．1 Effects of Low-Dose Prenatal Irradiation on the Central Nervous System in Mouse (■) Cell Migration in Cerebral Cortex After Chronic Irradiation during the Embryonic Day 14 to 17.

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Keywords: mouse brain, neuronal migration, prenatal chronic irradiation

It is well known that the development of the cerebral cortex in mammals can be severely affected by exposure to ionizing radiation at critical periods during gestation. Quantitative studies of low dose radiation effects on migration of neuronal progenitor cells in the cerebral mantle are required in experimental animals in order to contribute to pathogenic analysis of mental retardation seen among A-bomb survivors exposed in utero. In particular, long-term effects on neuronal allocation in adult animals after prenatal irradiation at different neurogenic stages should be evaluated. We previously reported the effects of acute exposure of developing brains to radiation at embryonic day 14 (E14) in mice. In the present experiments, we investigated effects of continuous exposure to radiation on the migration of neurons from the matrix cell zone towards the neocortical plate in mice during the period of the embryonic days 14 to 17.Pregnant mice (C57BLxC3H) received intraperitoneal injection of bromodeoxyuridine (BrdU), 0.5 mg dissolved in 0.5 ml saline／mouse on E14 of pregnancy to label S phase cells. The mice were then irradiated continuously with γ- rays at dose rates of 0.1 Gy per day, 0.3 Gy per day and 0.94 Gy per day from E14 to E17 by exposing them to a 137Cs source of 370 GBq at different distances. Brains from some embryos on E17 and some of the offspring at 3 and 8 weeks after birth from irradiated and control dames were either immersed or transcardially fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Four mm paraffin sections of the parietal cortex were processed for immunohistochemical examination of BrdU-labeled cells, using monoclonal anti-BrdU antibody (Becton Dickinson) followed by the peroxidase reaction visualized with 3,3'-diaminobenzidine. Immunostained sections were carefully observed under a light microscope and the locations of BrdU-labeled cells were plotted onto tracing paper using a camera lucida apparatus.BrdU-labeled cells were densely accumulated in the deeper portion of the ventricular zone of E14 mice neocortex. At E17 more than 70% of all of the BrdU-labeled cells were seen in the cortical plate of non- irradiated animals. In animals at 3 and 8 weeks after birth, most of the labeled cells (approximately 70%) were located in layer IV, while some of the cells were located in layers ■／■ or in layers ■／■. Chronic exposure to γ-rays with dose-rates of 0.1 and 0.3 Gy／day from E14 to E17 did not affect the initial migration of neurons in developing the neocortex and relative distribution of BrdU-labeled cells in cerebral cortex in young and mature mice. After continuous irradiation with dose-rate of 0.94 Gy／day for 3 days, decelerated migration of neurons was observed during the period of embryonic development and some difference of distribution pattern of BrdU-labeled cells in mature cerebral cortex was found between prenatally irradiated and control mice. We have reported previously

decelerated migration of neurons in neocortex during the embryonic period and aberrantly placed neurons in the cerebral cortex in young mice after acute prenatal x-irradiation with dose of less than 1 Gy at E14. From the present results, on the contrary, it seems that deleterious effects of prenatal radiation to development of murine cerebral cortex are reduced by chronic low dose- rate irradiation.

3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．2 Dose-Response Relationship for Induction of Hepatocellular Tumors in Mice Irradiated Neonatally with Gamma Rays

Shunsaku Sasaki

Keywords :hepatocellular tumors, gamma rays, dose-response relationship, mice, neonatal period

Our previous studies demonstrated that mice of the early postnatal period are highly susceptible to induction of hepatocellular tumors. In this report we show the dose-response relationship for induction of hepatocellular tumors which was obtained using a new method. Data for analysis were obtained by a lifetime experiment using a total of 2,978 female B6C3F１ mice. Animals were irradiated at day 0 of the neonatal period with 0.48, 0.95, 1.43, 1.90, 2.38, 2.85, 3.80 or 5.70 Gy gamma rays from 137Cs. After natural death, macroscopic and microscopic examinations were carried out for each mouse.Incidences of hepatocellular tumors are plotted against dose of gamma rays in Fig.1.

Tumor incidences in all irradiated groups were significantly higher than that in the control group. The shape of the dose-response relationship may be described as follows: 1) rapid increase in incidence in dose range up to 1 Gy; 2) gradual increase in dose range between 1 to 3 Gy; 3) gradual decrease in dose range over 3 Gy. Dose-response relationship for induction of hepatocellular tumors was fitted to a model which was expressed as the equation: $I(D) = I(0)(1+aD)\cdot(1-bD)\cdot\exp(-cD)$ where I(D) represents incidence of tumors in a group irradiated with dose D, I(0) incidence in the control group, parameter a the coefficient for induction of tumors, parameter b the coefficient for decrease in incidence by competing risks, and parameter c the coefficient for killing of the potentially tumorigenic cells. From regression analysis, values of the parameters were estimated asfollows: $a=1.739\pm0.135$, $b=0.120\pm0.002$, $c=0.082\pm0.015$. Dose-response relationship for induction of hepatocellular tumors seems to be well described by the equation. This model may be useful for analysis of dose-response relationship for induction of other tumors.

Fig.1. Dose-response relationship for induction of hepatocellular tumors in female B6C3F１ mice irradiated neonatally with gamma rays.

3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．3 High LET Radiation-Induced Tumors In Mice : Tumor Spectrum and RBEs

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Keywords:high LET radiation, radiation carcinogenesis, tumor spectrum, RBE, life shortening

It has been reported that incidence of liver tumors increased slightly among A-bomb survivors. On the other hand, many cases of radiation therapy using high LET radiation, such as fast neutrons, fission neutrons, protons, and other heavy ion beams, have been carried out during the past 20 years. There is now a fear that secondary tumors may have been induced in those who received high LET radiation therapy. We are studying the delayed carcinogenic effects of low doses of neutrons on a strain of mice relatively resistant to the carcinogenic effects of radiation .C57BL／6J male mice (4 weeks of age) were supplied by the Laboratory Plants and Animals Section of our institute. They were maintained under SPF conditions. In this study, each experimental group of mice received an examination to determine the effect of the two types of whole body irradiation on life span and carcinogenesis during their life span. Mice were housed in lucite cages with a micro-organismic barrier system that enabled the mice to breath sterilized air during neutron irradiations.Cyclotron-accelerated deuterons up to a mean energy of 30MeV were bombarded on to a thick 10Be target. It radiated neutrons with a mean energy of 13MeV and an averaging LET of 10.7keV／μm. Gamma-ray contamination was estimated to be less than 4% of the beams. The dose rate was 33cGy／min.Daily death checks were carried out, and ordinary autopsies and histological examinations were made on all mice. The life shortening effect of the radiation was analyzed by a life shortening ratio calculated from 50% survival periods obtained from survival curves using the Kaplan-Meire's method.All groups of mice and the numbers of their tumors observed are shown in Table 7. The fast neutrons induced a wide variety of tumors, including tumors of the endocrine organs, such as thyroid gland, pituitary gland, and adrenal gland, and soft tissues. Two to five different tumors often occurred simultaneously in one mouse, especially in fast neutron irradiated-mice. Tumor incidence is shown in Table 2. as crude incidence and age-adjusted incidence for each group. The life shortening effect of the radiation was analyzed by z-tests and generalized Wilcoxon tests. Fifty percent survival periods, estimated by the Kaplan-Meier's method, and life shortening effect of these two kinds of radiation on each group(life shortening ratio) are also listed. The effect of gamma-rays was lower than the effect of neutrons., and RBE was calculated as 2.4 for the neutrons. Dose dependent increases in liver tumors were observed in the groups irradiated with 0.125, 0.25, 0.5 and 1Gy of neutrons (Table 2).

Higher incidences than that of the control group were observed in the 0.25 and 0.5Gy neutron groups. In this study remarkably higher incidences of liver tumors and a linear increase of the tumors were observed in the mice that received the low dose range of whole body neutron irradiation than in the mice irradiated with gamma-rays. The difference in the dose response between the two kinds of fast neutrons was small. We calculated the RBEs from the slopes of the initial rising curves of these dose response curves as 33 for 2MeV fast neutrons and 24 for 13MeV fast neutrons in our previous study, in which data from low dose

gamma-ray irradiated groups was not yet available. We recalculated the RBE using these new data as 33 for 13MeV fast neutrons. RBEs concerning liver tumor were comparable between the two kinds of neutrons.

3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．4 Effects of Calorie Restriction on Hepatoma in C3H／He Mice

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Keywords: hepatoma, calorie restriction

Host-defense mechanisms from cancer are known to be modulated by changing an environmental factor. The spontaneous incidence of myeloid leukemia is about 1% in C3H／He male mice, and the incidence increases up to 23.3% when whole-body irradiation is given with 3 Gy of X-rays. However, such radiation induced-increase of myeloid leukemia is significantly decreased by calorie restriction(CR), i.e., 7.9% and 10.7% when CR was started before irradiation(6 weeks old) and after irradiation (10 weeks old). It is well known that C3H／He mice develop hepatoma spontaneously with high incidence. Then this report examined whether such spontaneous tumors would also be decreased by CR. Diets consisted of four different calorie controlled regimens, 60, 65, 70 and 95Kcal per week per mouse. The calorie-intake was adjusted by varying the amount of carbohydrate and dextrose, but giving a constant amounts of other nutrients, such as protein, lipid, vitamins and minerals. The body weight of mice was measured weekly. Mice in the restricted groups were controlled to keep their body-weight between 25 ～ 27g by an appropriate combination of the four different calorie diets, so that mice were fed with the least-sufficient amounts of calories to maintain normal growth. Animals were placed in 6 groups : the control diet without radiation (CC), the control diet with irradiation at 3Gy (3C), the restriction A group with (3RA) or without (CRA) irradiation, the restriction B group (3RB)with or without (CRB) irradiation. Control diet groups were fed the 95Kcal diet from 6 weeks old over their life span. Restriction A groups were also fed the 95Kcal diet from 6 to 10 weeks old. Restriction B groups were fed the 65Kcal diet, also from 6 to 10 weeks old. Mice in the two restricted groups had their body-weight controlled according to above described procedures.The incidence of hepatoma in both control-diet groups, CC and 3C, were 70% and 68%, respectively. On the other hand, the incidence of hepatoma in all groups for calorie restriction significantly decreased, i.e., 31%, 37%, 36% and 51% in CRA, 3RA, CRB and 3RB, respectively. The calorie restriction not only reduced the incidence but also reduced the latent period of the hepatoma (Figure1). In the control-diet groups (CC and 3C), the first hepatoma appeared at ages of 406 and 349 days, respectively, whereas, in restricted groups, it appeared at 586 days in CRA and 526 days in 3RA. Interestingly, the incidence of hepatoma was not changed with radiation, however, the onset of hepatoma in all diet groups was promoted by radiation. The calorie restriction reduced not only the myeloid leukemia, but also spontaneous hepatoma.

Fig.1 Cumulative incidence of hepatoma.

[Publications]

3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．5 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

Shigeru Kobayashi, Hiroshi Otsu, Yuko Noda and Toshiaki Ogiu

Keywords:lung tumors, nude mice, urethan, gamma-ray irradiation, tumor acceleration

The role of immunological surveillance in carcinogenesis is still controversial. In our previous experiments, urethan- lung tumorigenesis in athymic (nu／nu) mice and euthymic (nu／+) littermates was examined, and it was concluded that immunosurveillance mediated by T-cells could not be demonstrated. However, the reported enhancement of development of various tumors by ionizing radiation might be achieved through modulating host immunological conditions. In the present experiment, nu／nu and littermate nu／+ mice were treated with 1 to 4 Gy of gamma-rays alone at 6 weeks of age or treated with 0.5 mg／g body weight of urethan at 14 days followed by 1 to 4 Gy of gamma- rays 4 weeks later. Lung tumors were assessed at 6.5 months of age. Ionizing radiation itself caused a very low incidence of these lesions. On the other hand, multiplicities and incidences of lung tumors after the urethan treatment were similar between the two phenotypically different groups of mice (1.66 and 1.84 tumors／mouse, 73 and 80% incidences, for nu／nu and nu／+ cases, respectively). This urethan-lung tumorigenesis was significantly enhanced by gamma-rays in both nu／nu and nu／+ mice, and the magnitude of tumor enhancement was somewhat higher in nu／

+ mice than in nu／nu mice, especially with 2 Gy dose. In conclusion, the lung tumorigenicity of gamma-ray irradiation itself and the enhancing effect of radiation on urethan-induced tumorigenesis are scarcely influenced by the immuno- surveillance mechanism mediated by T-cells.

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3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．6 Bone Damages in Hind Limb by X-ray Irradiation to Parathyroid and Thyroid in Young Rats

Satoshi Fukuda and Haruzo Iida

Keywords: x-ray irradiation, bone damage, histomorphometry, parathyroid-thyroid, rat

Bone damages which occurred after local X-ray irradiation to the neck, including the parathyroid and thyroid, in young male rats were examined and compared to those in groups irradiated to the whole body with dose of 1.25 - 5 Gy, and locally to the hind limb with doses of 5 -15 Gy at the age of 4 weeks. Five months after irradiation, the bone and serum samples were analyzed. Serum PTH level decreased significantly up to 7.5 Gy and calcitonin level was less than the detectable limit over 5 Gy. There was no significant difference in the ionized calcium level. High correlations were seen between doses and individual values such as the bone length, strength and calcium content in the femur, the values decreased significantly in the whole body irradiation group over 2.5 Gy, and those in the hind limb irradiation group at 7.5 - 15 Gy. In the histomorphometric analysis of the secondary spongiosa area in the proximal metaphysis of tibia, bone volume and trabecular thickness had a tendency to decrease up to 5 Gy in the neck and whole body irradiation groups, but to increase over 5 Gy, for the neck as well as whole body and hind limb irradiation groups.

Mineral apposition and bone formation rates had a tendency to decrease with the increase of dose and decrease significantly over 7.5 Gy in the hind limb irradiation group. The results indicate that irradiation to the neck locally as well as the whole body in young rats induces damages in bones such as mineral loss and fragility at less than 5 Gy, and all of these advance with morphologic changes such as atrophy over 5 Gy.

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3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．7 Bone Histomorphometric Analysis in the Dahl-Iwai Salt-sensitive Rat (DIS／Eis)

Satoshi Fukuda, Haruzo Iida, Kazuto Yamazaki＊ and Tsuneo Wakabayashi＊ (＊Eisai Co., Ltd)

Keywords:bone histomorphometry, Dahl-Iwai salt-sensitive rat, ospetopenia, osteoporosis

Effects of salt loading on the bones in Dahl-Iwai salt sensitive (DIS／Eis) and resistant (DIR／Eis) rat were examined. Both strains were divided into two groups, and fed on 8.0 % and 0.3 % salt diets for 6 weeks after being fed on a 0.3

% salt diet until 5 weeks old. In the 8.0 % salt diet group of DIS／Eis rats, there were significant differences in the decrease of body weight, elevation of systolic blood pressure, increase of excreted calcium in urine, decrease of alkalinephosphatase activity, and also in the results on histomorphometric examination for the secondary spongiosa area of undecalcified tibial proximal metaphysis, decrease of bone volume, trabecular number and osteoid volume, and increase of trabecular separation, as compared to those in 0.3 % salt diet group of DIS／Eis (Fig.1). In the DIR／Eis rats , no significant changes were seen, except for the increase of calcium in plasma between 0.3 % and 8.0 % salt diet groups. These results indicate that excessive salt intake is accompanied by osteopenia or osteoporosis and hypertension in rats having a genetic salt sensitive factor.

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Fig.1. Bone volume／tissue volume(BV／TV) in each group.

3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．8 Intestinal Calcium Absorption and Response of Calcium Regulating Hormones in Stroke- prone Spontaneously Hypertensive Rat (SHRSP) as a model of Osteoporosis

Satoshi Fukuda, Satoru Tsuchikura, Haruzo Iida, Katsumi Ikeda＊, Yasuo Nara＊ and Yukio Yamori＊ (＊Kyoto Univ.)

Keywords:intestinal calcium absorption, SHRSP, osteoporosis

Because of low levels of serum calcium (Ca) in SHRSP, intestinal Ca absorption and urinary Ca excretion were examined. Intestinal Ca absorption rate from diets was lower in SHRSP than in the normotensive WKY(Wistar Kyoto) and WM(Wistar Mishima) strains at all ages. Urinary Ca excretion was 3-5 times greater and increased with age in SHRSP. With oral administration of vitamin D, the intestinal Ca absorption rate increased significantly in SHRSP at 6 months and in WKY at 12 months. The urinary Ca excretion increased significantly in WKY and SHRSP at 6 months and in WKY at 12 months. Absorption rates for various chemical forms of Ca were lower in SHRSP than in WKY and WM, and decreased in the order of oxide ＞ carbonate ＞ chloride in SHRSP and WM rats. The absorption rate of CaCl2 was lower in SHRSP than in WM. Ca transport rate was lower and increased with age in SHRSP. Serum Ca levels elevated significantly 15 min after pCa injection in both TPTX and intact groups and the levels were TPTX ＞ intact, WM ＞ SHRSP, but these did not change in the intact group and were lower than the base line in the TPTX group after CaCO3 injection in SHRSP and WM rats. In the intact SHRSP group, PTH levels were suppressed, decreasing rather significantly ($p<0.001$), by pCa at 6 and 12 months, while the levels did not change at 6 months and decreased slightly at 12 months for CaCO3. Calcitonin levels were elevated significantly for CaCO3 ($p<0.05$) at 6 months and pCa ($p<0.001$) at 6 and 12 months. Serum Ca levels after intravenous injection were lower in SHRSP than in WM and decreased with age in SHRSP. The results indicate that SHRSP has lower intestinal Ca absorption and higher urinary Ca excretion, but with a latent function for intestinal Ca absorption which is able to respond to Ca regulating hormones for Ca loading in the aged.

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3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．9 Prevention of Cilia-Associated Respiratory Bacillus by Antibiotics and Its Diagnosis by Polymerase Chain Reaction

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Keywords:CAR bacillus, antibiotics, sulfamerazine, RT-PCR

The cilia-associated respiratory (CAR) bacillus, a gram-negative filamentous bacterium, was identified in 1985. Since then it has been recognized as one of the causative agents of murine chronic respiratory disease (CRD). Recent reports have shown that CAR bacillus may be potentially widespread in many species of la-boratory and domestic animals. However, little is known yet concerning this disease. The present studies describe prevention and eradication by treatment using antibiotics (Experiment A) and diagnosis by the reverse transcription (RT)-polymerase chain reaction (PCR) (Experiment B). Experiment A: Specific-pathogen-free female BALB／c mice, 10 to 11 weeks old, were used. The mice were orally administrated sulfamerazine, ampicillin, and chlortetracycline at a rate of 500 mg／L of drinking water. They were infected by intranasal inoculation with 10^6 bacilli of the SMR strain of CAR bacillus, and treated with the antibiotics starting 1 week before, 1 week after, or 4 weeks after the inoculation, for 5, 3, or 4 weeks respectively, then were examined. Only the mice administered sulfamerazine starting 1 week before the inoculation had no antibody titers to the bacilli and no pathologic respiratory tract lesions or bacterial colonization. These findings suggest that prevention and eradication of CAR bacillus infection is possible by treatment with sulfamerazine. Experiment B: Experimentally infected female Jcl: ICR mice, 4 weeks old, and naturally infected rats were used. In the experimental infection, the mice were in contact with infective mice previously inoculated with $7.5X10^5$ bacilli of the CB-M strain. The designs of primers and probe for RT-PCR amplification were based on the 16S rRNA sequences described previously. CAR bacillus was detected in oral swab samples from mice by RT-PCR on day 3 post-contact infection. In the naturally infected rats, infectious rate by RT-PCR corresponded to serum antibody-positive rate by enzyme-linked immunosorbent assay. These findings suggest that the RT-PCR is a specific, highly sensitive and reliable procedure for detecting CAR bacillus in mice and rats.

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3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．10 Enhanced Metastasis After Local Irradiation to a Murine Neuroblastoma

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Keywords:liver metastasis, surgery, N-methylformamide

This study was carried out,using a murine model, to determine the effects of local therapy by either surgery or local irradiation on the enhancing pattern of distant metastasis. Transplanted C-1300 neuroblastomas in hind legs of syngeneic mice were treated either by surgery or local irradiation. These local treatments had adverse effects on establishment of distant metastasis. Liver metastasis was found 14 to 18 days after local treatment,but the tumors without treatment did not develop metastasis. Metastatic incidence depended on the size of the primary tumors: amputation of hind legs with primary tumors 9 or 12 mm in diameter produced more liver metastasis than amputation of legs with tumors ≦ 7 mm in diameter. Radiation doses ranging from 22 to 51 Gy completely eradicated 7-mm tumors, but failed to reduce liver metastasis. When systemic drug therapy with N-methylformamide, a maturational agent, was combined with amputation, a remarkable reduction was observed in the number of liver metastases.

However, local control by irradiation combined with N-methylformamide treatment did not have any effect on the incidence of liver metastasis. The ineffectiveness of local control by radiotherapy on distant liver metastasis in this neuroblastoma animal model suggests that primary tumors, even those which diminish after irradiation, might keep supplying tumor cells with enhanced metastatic ability toward distant metastatic sites.

[Publications]

Iwakawa, M., Ando,K., Koike,S., Chen,Y. -J., Okudaira, R. and Ohkawa, H. :Int. J. Pediatric Hematol／Oncol. 2, 433-439, 1995

3．4 BIO-MEDICALSCIENCE Pathology and Physiology

3．4．11 Mouse Skin Reaction after Fractionated Irradiations with 290 MeV／u Carbon Ions

Koichi Ando, Sachiko Koike, Yu-Jau Chen, Kumie Nemoto, Soichiro Ando, Nobuyoshi Kobayashi, Tohru Ohbuchi, Wakako Shimizu and Tatsuaki Kanai

Keywords:skin reaction, LET, recovered dose, Fe plot

Use of heavy particle radiotherapy for malignant disease has recently started in Japan employing carbon-12 beams produced by the HIMAC synchrotron. Current clinical trials of phases ■ and ■ use a standard protocol of 18 fractions in 6 weeks with dose escalation. This protocol is based on some biological findings and past experience in our institute using fast neutrons. However, the optimum fractionation schedule must be established for more effective treatment. We have investigated carbon-dose responses of skin reactions after daily fractionations to the mouse leg (Fig.1). Irradiations with 1, 2 and 4 fractions indicated that the isoeffective doses for radiation with LETs lower than 20 keV／μm increased with an increase in the number of fractions, but not the isoeffective dose for radiation with 100 keV／μm. The recovered dose from 1 to 2 fractions reached a maximum at 40 keV／μm while the recovered dose per fraction from 2 and 4 fractions decreased with an increase in the LET. The Fe plot was linear at the LETs lower than 20 keV／μm and at the LET of 100 keV／μm, but curved slightly at the LETs between 40 keV／μm and 80 keV／μm. These results suggest that the Fe plot is not valid at intermediate LETs of 40 keV／μm through 80 keV／μm.

Fig.1 Isoeffective doses of carbon ions to produce an averaged skin reaction score of 2.5. LETs less than 20keV／μm were located at the entrance plateau while those than 40 keV／μm were located at the 6■cm higher Spread■Out■Bragg■Peak.

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．1 Allelic Losses at the APRT Locus in Human Cells

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Keywords: adenine-phosphoribosyltransferase, 2,6-diaminopurine resistance, LOH, RFLP, microsatellite locus, mitotic recombination

Constitutional loss or inactivation of one copy of a tumor suppressor gene, as exemplified by hereditary retinoblastoma, increases the propensity for malignancy by reducing the number of events required for the complete loss of the negative regulatory function. An immortalized B-lymphoblastoid cell line, WR10, derived from an obligate heterozygote of hereditary 2,8- dihydroxyadenine urolithiasis, adenine phosphoribosy ltransferase (APRT) deficiency, has enabled us to develop an assay system for dissecting the second step in loss-of-function mutations, i.e. the forward mutation from APRT +／- to APRT -／- or APRT o／-, and for determining the potency of physical and chemical agents to produce such mutations. The non-functional APRT allele on chromosome 16 (16q24.3) in WR10 cells bears a nonsense mutation in the exon 3. WR10 was found to be also heterozygous for a SphI RFLP associated with the gene, which allowed the functional and the constitutionally non-functional alleles to be distinguished by Southern blot analysis using an APRT probe.

The base-line frequency of cells resistant to 100 μM of 2,6-diaminopurine (DAP) was found to be 1.1 x 10-5 with a mutation rate of 1.65 x 10-6 ／ cell ／ generation. Recloned DAP r mutants, in general, grew as rapidly as wild-type WR10 cells (population doubling time, 36 h). Exposure of WR10 cells to γ-rays resulted in a dose-dependent increase of DAPγ mutant fraction up to 2.5 x 10-4 at 2 Gy, whereas induced mutant fraction was 4.7 x 10-5 for 6-thioguanine resistance (TG r) with the base-line frequency of 1 x 10-6. A substantial proportion of the spontaneously-arising mutants (22／26, 85 %) and virtually all of the γ-ray-induced mutants (64／69, 93 %) lost the functional allele, judging by loss of heterozygosity (LOH). Dosage blotting revealed that about half of the spontaneously-arising and γ-ray-induced mutants with LOH showed a reduction to homozygosity of the mutant allele, implying that the mutated allele was duplicated due to mitotic recombination or gene conversion. Non-disjunction with reduplication of the mutant chromosome 16 was ruled out by the retention of heterozygosity at 16p microsatellite loci in all of the mutants tested with LOH at the APRT locus. About 70 % of mutants, both those arising spontaneously and those induced by radiation, showed LOH at the D16S265 (16q21) and the D16S308 (16q12.2) loci on the long arm. The distribution of the sites for somatic recombination or for deletion breakpoints in radiation-induced mutants was indistinguishable from that in spontaneously-arising mutants. These results suggest that somatic recombination and／or deletion occur frequently close to the border between the heterochromatin and the euchromatin regions on the chromosome 16q.

[Publications]

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．2 Hypomutability by γ-rays of Thymic Lymphoma Cells Derived from a SCID Mouse

Ikuko Furuno-FukushiI, Kouichi Tatsumi, Ikuko Furuno-Fukushi, Kouichi Tatsumi, Masahiro Muto, Hirobumi Teraoka＊, Fumiaki Watanabe and Toshiaki Ogiu (＊Tokyo Medical and Dental Univ.)

Keywords : SCID mouse, γ-rays, mutant induction, Hprt, DNA dependent PK

The SCID mutation affects both V(D)J recombination and DNA double- strand break repair. To get an insight as to the relationship between DNA double-strand break repair and mutagenesis, we determined survival and mutation for 6-thioguanine resistance as a function of dose of γ-irradiation in SCA1 cells that were derived from a spontaneous thymic lymphoma in a SCID mouse. SCA1 cells were very sensitive to the cytotoxic effect of γ-rays and D0 value was estimated as 0.25 Gy. 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. The mutant fraction at the Hprt locus after γ-irradiation was very low, i.e. irradiation of SCA1 cells at the dose as high as that which gave 10% survival yielded only twice as high a mutant frequency as the spontaneous mutant fraction. After exposure to UV-light, SCA1 cells showed an essentially normal profile in the dose survival curve. Mutant fraction following UV irradiation increased linearly against UV dose in SCA1 cells. These results suggest that SCID mutation renders mouse cells hypomutable by γ-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.

[Publications]

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．3 Heritable Malformations in the Progeny of the Male Medaka (Oryzias latipes) Irradiated with X-rays

Yuji Ishikawa and Yasuko Hyodo-Taguchi

Keywords: genetic defects, malformations, x-ray irradiation, development, medaka, fish

Heritable malformations were examined in the progeny of x-ray irradiated male medaka (Oryzias latipes) by three-generation crosses. Two x-irradiated male fish were pair-mated with nonirradiated females to produce F 1 founders, and each F 1 fish was pair-mated with a nonirradiated fish to produce F 2 progeny. For detection of recessive mutations, pair-matings between F 2 siblings were performed for each F 1 family. Morphogenesis of the embryos of each generation was observed using a stereomicroscope, throughout the entire period of embryonic development, for identification of anomalies. In the F 1 embryos, the frequency of dominant lethals, which were accompanied by various types of malformations, was increased by the X- irradiation. Two out of 30F 1 pairs produced a number of malformed and lethal F 2 embryos, indicating inheritance of high rates of the dominant lethals in the two F 1 families. Moreover, F 2 sib-pairs offspring of which exibited high rates of dominant lethals were found in ten out of 28 F 1 families. Recessive lethal mutations, which were associated with a particular phenotype, were found in two out of the 28F 1 families. These results indicate that the heritable malformations induced by X-irradiation can be studied in the medaka.

[Publications]

Ishikawa, Y. and Hyodo-Taguchi, Y.: Mutation Res. (in press).

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．4 Production of Germfree Mouse by Embryo Transfer

Masanori Okamoto and Tsuneya Matsumoto

Keywords: embryo transfer, ermfree mouse

Conventionally, in preparing germfree (GF) mice, the uterus is removed from animals immediately before delivery, the fetus is taken out, and nursed by a GF foster mother. With this tehnique, it is difficult to determine the optimum time to remove the uterus. The resuscitation rate of offspring can be reduced if natural delivery occurs earlier than expected or if surgical delivery is too early. Furthermore, foster nursing requires skill. To increase the efficiency of GF mouse production, we examined the embryo transfer technique.

Mature female Jcl:MCH (ICR) mice served as donors for embryo collection after they were mated with mature males of the same strain. GF female C3H／HeMS mice, which had been maintained at our facility, served as recipients after having mated with males of the same strain previously vasectomized. The procedure for producing GF mice, by embryo transfer, is outlined in the flow chart in Fig.1. GF C3H male mice underwent a vasectomy aseptically within a clean bench. Infertile copulation between vasectomized male and female C3H mice was then induced to produce pseudopregnant recipients. Superovulation was induced with 5 IU of PMSG and hCG injected 48 h apart in conventional female ICR mice, which were then mated with males . On Day 4 post-mating, morphologically normal morula and blastocyst embryos were collected, aseptically, from these females in a clean bench. The collected embryos were immediately transferred, aseptically, into the uteri of the recipients, which had been moved, via a sterile lock, into the clean bench, on Day 3 of infertile copulation with sterile males. The recipients were returned to the vinyl isolator and underwent pregnancy, delivery and nursing. A sterility test was performed, according to the methods recommended by the Japan Experimental Animal Research Association using a combination of TGC and CM medium, or GAM medium. To confirm that embryos had been collected aseptically, the embryos collected from a superovulated donor mouse were put into culture medium. One half of the embryo-containing culture medium was then combined with one test medium and the other half was combined with the other test medium. These two test media were then incubated at 20 and 37℃ for 2 weeks. The sterility test of recipient mice was confirmed by checking their feces 4-5 days after embryo transfer and after delivery. The flexible vinyl isolator was also examined for sterility twice, when examining the recipient mice. The newborns were examined for sterility when they were weaned at the age of 3 weeks.

From the 3 donor mice, we collected 16, 13 and 15 morphologically normal morulae and blastocysts, respectively. These embryo-containing culture media were inoculated into each test medium, and incubated for 2 weeks. All culture media containing embryos were found to be sterile. From donor mouse, we collected 12 morphologically normal morulae and blastocysts. All embryos were transferred to recipients and 6 newborns were delivered. The feces samples, collected from the 2 recipients 4-5 days

after embryo transfer and after delivery, were sterile. All feces samples from the 6 weaned animals were also sterile. The flexible vinyl isolator was also sterile in both tests.

Hysterectomy has conventionally been used at our facility to produce GF mice. The success rate using this technique was about 20% before 1989 and approximately 45% in 1990 and 1991. The low success rate of this technique is attributable to the difficulty in determining the optimal timing for surgical delivery and in the nursing of newborns by the foster mother. By our new method, the recipient mice, to which the embryos have been transferred, can deliver and nurse the neonates without human intervention. Furthermore, our technique of embryo transfer allows the animals infected by pathogens to be cleansed by transferring their embryos into clean recipient mice.

The present study, indicated that GF mice could be produced by embryo transfer, in addition to the conventional techniques of hysterectomy and cesarotomy. Furthermore, we developed new aseptic techniques for producing vasectomized sterile males from GF mice and for manipulating embryos. We connected the flexible vinyl isolator, which accommodated GF mice, to the clean bench for aseptic surgery. A follow-up study will be performed using a larger number of animals and also to establish a more efficient, more practical technique for producing GF animals. This method of producing GF animals, in combination with reproductive biotechnology such as techniques for storing frozen embryos, should be useful for strain maintenance, transportation of mice, and in vitro fertilization. Fig.24. Experimental procedures for production of germfree mice using an embryo transfer technique. SL: Sterile lock.

[Publications]

Okamoto, M. and Matsumoto, T.: Proceedings of the XIIth International Symposium on Gnotobiology, in press.

3．5．5 Comprehensive Cloning of Schizosaccharomyces pombe Genes Encoding Translation Elongation Factors

Kazuei Mita, Mitsuoki Morimyo, Koichi Ito＊, Kimihiko Sugaya, Kanae Ebihara＊, Etsuko Hongo, Tomoyasu Higashi, Yasuko Hirayama and Yoshikazu Nakamura＊ (＊Univ. of Tokyo)

Keywords: nucleotide sequence, cDNA catalog, fission yeast, translation elongation factor

Our purpose in the cDNA project using fission yeast Schizosaccharomyces pombe is to compile a comprehensive list of the house-keeping genes of eukaryotes. Since most of the house-keeping genes are conserved throughout eukaryotes and the fission yeast is the simplest eukaryote with a minimum number of essential genes of a eukaryotic cell, we chose it as a model organism to understand the function of house-keeping genes. We have, thus far, sequenced more than 12,000 clones and identified more than 2,500 independent clones.

In the course of theSchizosaccaromyces pombe cDNA project, we succeeded in cloning all the genes encoding translation elongation factors EF-1a, EF-1b, EF-1g, EF-2 and EF-3. With the exception of the EF-1g gene, the nucleotide sequence of S. pombe elongation factors has not been previously reported. For EF-1a, we found three genes whose amino acid sequences are quite homologous to each other (99.5%), but whose 3'UTRs (untranslated region) are completely different. Southern blot indicated that those three EF-1a genes are located at different loci. Northern analysis indicated that one of these EF-1a genes was inducible with UV-irradiation. The amino acid sequence predicted from the nucleotide sequence of the S. pombe EF-1b cDNA clone covered almost all the CDS (coding sequence) of EF-1b except the first methionine which has 55.4% identity with that of S. cerevisiae. We also identified two copies of S. pombe EF-2 genes. Their amino acid sequences deduced from nucleotide sequences are identical (100%), but they have different 3'UTRs. The location of these two EF-2 genes in different loci was proved by Southern analysis. The S. pombe EF-3 cDNA clone encoded only a third of the CDS from the C-terminal and its deduced amino acid sequence has a 76% identity with those of other yeasts and fungi. Table 1 summarizes the translation elongation factors of S. pombe identified by the cDNA project.

[Publications]

Mita, K., Morimyo, M., Ito K., Sugaya, K., Ebihara, K., Hongo E., Higashi, T., Hirayama, Y. and Nakamura, Y.: Gene, in press.

3．5．6 Organization of NOTCH4 and (CTG) n Polymorphism in the Human Counterpart Gene of Mouse Proto-Oncogene int-3

Kimihiko Sugaya, Shun-ichi Sasanuma, Junko Nohata, Terumi Kimura, Tatsuo Fukagawa 1 , Yasukazu Nakamura 1, Asako Ando 2, Hidetoshi Inoko 2, Toshimichi Ikemura 1, and Kazuei Mita (1 National Institute of Genetics, 2 Tokai Univ.)

Keywords : gene structure, variable number tandem repeat, phylogenetic relationship, chromosome duplication, major histocompatibility complex

The cDNA and genomic clones for the human counterpart of mouse mammary tumor gene int-3 were isolated and sequenced. We designated this human MHC class ■ gene as NOTCH4 since very recently the complete form of the mouse proto-oncogene int-3 has been clarified by sequencing for cDNA clones and named Notch4. The present human NOTCH4 sequence is the first example of the genomic sequence for the extracellular portion of the mammalian Notch4, and the exon／intron organization was clarified by comparing with the mouse Notch4 cDNA sequence (Fig.1). The comparison of the predicted amino acid sequence of human NOTCH4 with sequences of other Notch homologues of a wide range of species including mouse Notch4 revealed four subfamilies for mammalian Notch. In the protein coding region of human NOTCH4, we found (CTG)n repeats showing VNTR polymorphism for different HLA haplotypes. We also found ten genes mapped on 6p21.3, including NOTCH4, which have counterparts structurally and functionally similar to those mostly mapped on 9q33-q34, indicating segmental chromosome duplication during the course of evolution.

Fig.25. Gene organization of human NOTCH4. (A) Location and structure of NOTCH4 gene. Black boxes on the upper line indicate genes found in the junction area between MHC classes ■ and III by our group, and two cosmid clones (KS74 and KS71) harboring the NOTCH4 sequence are indicated below the line. The lower line indicates the EcoRI map near and at the NOTCH4 locus. Genomic sequences determined in this study and previously (f1-f7) are indicated by thick horizontal bars below the line; black boxes on the bars show exons. The exon including (CTG)10 repeats and initiator methionine ATG is indicated by an asterisk. A cDNA clone, PB5P4, is shown as a thick horizontal line, CDSs of genomic sequence as thin horizontal line and short vertical bars indicate sites for intron insertion. Regions corresponding to genomic sequences determined are connected by slant lines. (B) A schematic representation of functional domains of the NOTCH4 protein. The CDSs are predicted from open reading frames of genomic sequence and PB5P4 cDNA.

[Publications]

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．7 Identification and Characterization of a New Gene Physically Linked to the ATM Gene

Takashi Imai, Masatake Yamauchi, Naohiko Seki＊, Takehiko Sugawara, Toshiyuki Saito, Yoichi Matsuda, Hiroko Ito, Takahiro Nagase＊, Nobuo Nomura＊ and Tada-aki Hori (＊Kazusa DNA Res. Inst.)

Keywords: ataxia telangiectasia, ATM, NPAT, chromosome 11q22-23

Ataxia telangiectasia (AT) is an autosomal recessive disease of unknown etiology associated with cerebellar ataxia, oculo- cutaneous telangiectasia, immunodeficiency and hypersensitivity to ionizing radiation. Although AT has been divided into four complementation groups by its radioresistant-DNA-synthesis phenotype, the ATM gene has been isolated as the candidate gene responsible for all AT groups. We identified a new gene, designed NPAT, from the major AT locus on human chromosome 11q22-23. The gene encoded a 1,427-amino acid protein containing nuclear localization signals and phosphorylation target sites by cyclin dependent protein kinases associated with E2F. The messenger RNA of NPAT was detected in all human tissues examined, and its genomic sequence was strongly conserved through eukaryotes, suggesting that the NPAT gene may be essential for cell maintenance and may be one of the housekeeping genes. Analysis of the genomic region of NPAT surprisingly revealed that the gene existed only 0.5 kb apart from the 5' end of the ATM transcript with opposite transcriptional direction (Fig.1). The database search with the nucleotide sequence nestled between two genes indicated that this region contained a 256 bp sequence which was previously predicted to be one of the CpG islands isolated by Cross et al. Three degenerative TATA-like boxes were found in the upstream region at 782 bp, 893 bp, and 918 bp from the 5' end of the NPAT cDNA. Also, two GC boxes existed at 179 and 195 bp upstream of the NPAT transcript. Several types of transcriptional regulation motifs including one heat shock responsible element (102 bp apart from the 5' end of the gene) and two sites of the E2F binding domain (69 and 113 bp upstream) could be predicted in the 5' region of the NPAT gene which partly overlapped the first exon and intron of the ATM gene. It is noteworthy that some of the promoter elements and regulatory sequences work in either orientation. Therefore, this NPAT upstream region may regulate transcription of both genes.

These properties of the NPAT product and the chromosomal position suggest that NPAT, together with ATM, may be included in complex AT phenotypes.

[Publications]

Imai, T., Seki, N., Saito, T., Yamauchi, M., Matsuda, Y., Ito, H., Ogiwara, A., Nomura, N., and Hori, T.: DNA Res., 2, 113-121, 1995.

Imai, T., Yamauchi, M., Seki, N., Sugawara, T., Saito, T., Matsuda, Y., Ito, H., Nagase, T., Nomura, N. and Hori, T.: Genome Res. 6, 439-447, 1996.

Fig.1 Schematic diagram of the upstream region nestled between ATM and NPAT genes. The 5' untranslated region in the first exon of both genes is denoted by the white boxes. The first intron of both genes is represented by shaded bars. Possible E2F binding domain, SP1 binding sites, the heat shock responsible element or TATA-like motifs are marked by ellipses.

3．5．8 Isolation of 5' Portion cDNAs of ATM Gene for Ataxia Telangiectasia: A Large Open Reading Frame and Structural Heterogeneity of ATM Transcripts

Toshiyuki Saito, Masaki Kato, Masatake Yamauchi, Yoichi Matsuda, Takashi Imai, Takehiko Sugawara, Naohiko Seki＊ and Tada-aki Hori (＊Kazusa DNA Research Institute)

Keywords: ataxia telangiectasia, ATM gene, ATM transcripts

Ataxia telangiectasia (AT) is a hereditary disorder characterized by cerebellar ataxia, telangiectases, immune defects, a predisposition to malignancy and chromosomal aberration. These features are considered to be due to a defect in the G1-S checkpoint in AT cells after suffering DNA damage. Savitsky et al. (Science 268, 1749, 1995) reported a gene termed ATM from 11q22-23, the major AT locus, as a candidate gene for AT. The predicted protein product of ATM shared a homology with a signal transduction mediator, phosphatidylinositol 3-kinase of Saccaromyces cerevisiae. The described sequence of ATM cDNA in the report was, however, approximately a half of the transcript, implying the deduced amino acid sequence might be incomplete. We attempted to determine the whole structure of the gene product to investigate ATM function. A RACE (rapid amplification of cDNA ends) method was applied to isolate unknown 5' portion of the transcript and five types, at least, of ATM cDNAs were identified (Fig.1A). One type of them had the same structure of the reported in Science. Another of the other forms contained the largest open reading frame that might code for a relatively large, 351 kilodalton, protein consists of 3,057 amino acid residues (Fig.1B). The predicted protein contained a leucine zipper motif that suggests ATM protein may bind another protein (including ATM protein) and function in a protein complex form. Artificial expression of an anti-sense strand of the 5'-sequence of this ORF gave hyper sensitivity to X-ray irradiation to HeLa cells, indicating the protein encoded by the ORF would play a role in the responsive process to radiation-induced DNA damage of cells. The other three types of ATM cDNAs may derive from splicing-intermediate molecules or alternatively processed transcripts, requiring further study on heterogeneity of ATM transcripts. Biochemical analysis of ATM protein is now under way.

Fig.1 Structure of ATM gene product. A) Schematic representation of various ATM transcripts: Each bar represents the deduced protein from independently-isolated cDNAs. The top is from the report by Savitsky et al. White boxes represent the PI3 kinase domain. B) A putative large ATM protein: This protein is coded in the open reading frame of the third cDNA clone in A. The amino acid sequence written in bold letters is the newly isolated portion.

[Publications]

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．9 Comparative Genome Mapping of the Ataxia Telangiectasia Region in Mouse, Rat, and Syrian Hamster

Yoichi Matsuda, Takashi Imai, Tadahiro Shiomi, Toshiyuki Saito, Masatake Yamauchi, Hiroko Ito and Tada-aki Hori

Keywords: ataxia telangiectasia, comparative genome mapping, mouse

Ataxia telangiectasia (AT) is an autosomal recessive genetic disease characterized by pleomorphic clinical manifestations. At the cellular level, the AT cells exhibit a hypersensitivity to the killing effect of ionizing radiation and various abnormalities consistent with a defect involving DNA metabolism and／or maintenance of genomic stability. Recently, a candidate gene ATM was cloned and found to be mutated in AT patients from all complimentation groups, suggesting the possibility that the ATM gene is the sole gene responsible for AT phenotypes. To investigate the molecular pathology of this disorder, it is important to identify the mouse counterpart of the human AT gene. In vivo functions of the AT gene can be examined in a mouse model in which the mouse AT gene is targeted or knocked out.

In the present study, the chromosomal locations of the Atm, Acat1, and Rck genes in the mouse, rat, and Syrian hamster were determined by direct R-banding FISH using a rat cDNA fragment of Acat1 and mouse cDNA fragments of Atm and Rck as probes. Both Atm and Acat1 genes were colocalized to the C-D band of mouse chromosome 9, the proximal end of q24.1 of rat chromosome 8, and qa4-qa5 of Syrian hamster chromosome 12. These regions on the mouse and rat chromosomes have been identified as homologous to human chromosome 11q. To determine the order of genes in this region, fine genetic linkage mapping of the mouse AT region was performed using the interspecific backcross mice between (C57BL／6 x M. spretus) F１ females and M. spretus males. The Atm, Acat1, and Npat which is new gene physically linked to the ATM gene in human, and 12 flanking microsatellite DNA markers were examined. Fig.1 shows the gene order and recombination frequencies of each pair of loci examined in 150 backcross mice. No recombinations were found among the Atm, Acat1, and D9Mit6 loci, and these loci were mapped 2.0 cM distal to D9Mit99 and 1.3 cM proximal to D9mit102. Comparison of the linkage map of mouse chromosome 9 (MMU9) and that of human chromosome 11 (HSA11) indicates that there is a chromosomal rearrangement due to an inversion between Ets1 and Atm-Npat-Acat1 and the inversion of MMU9 originated from the chromosomal breakage at the boundary between Gria4 and Atm-Npat-Acat1 on HSA11. This type of inversion appeared to be conserved in three rodent species, mouse, rat, and Syrian hamster, using additional comparative mapping data with Rck gene. The present mapping information on MMU9 will facilitate consideration of the evolution of MMU9 and HSA11 and also be useful for construction of AT-model mice to investigate its gene functions in vivo.

[Publications]

Matsuda, Y., Imai, T., Shiomi, T., Saito, T., Yamauchi, M., Fukao, T., Akao, Y., Seki, N., Ito, H. and Hori, T.: Genomics, 34,347-352,1996.

Fig.1. Comparison of locations of homologous gene typed on MMU9 and HSA11.

The recombination distances between loci are shown in centimorgans (cM).

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．10 A Rare Fragile Site, FRA8E, Is Localized to Hereditary Multiple Exostosis Gene

Tada-aki Hori, Naohiko Seki＊, Toshiyuki Saito, Masatake Yamauchi, Hiroko Ito and Takashi Imai (＊Kazusa DNA Res. Inst.)

Keywords : chromosomal fragile site, FRA8E, 8q24.1, EXT1 gene

The rare fragile site is a specific point on a chromosome that is expressed as an isochromatid gap or break under certain conditions of cell culture, and is inherited in a Mendelian codominant fashion. Five folate sensitive fragile sites have been cloned and characterized. The molecular basis of these fragile site mutations was shown to be a new class of mutation,called dynamic mutation, due to an allelic expansion of (CCG)n repeats. The mechanism responsible for other types of rare fragile sites, i.e., distamycin A-inducible and BrdU-requiring, is unknown, although cytogenetic studies suggested that these fragile sites played a mechanistic role in breakage and recombination, and may also be integration and modification sites of foreign viral genome. A distamycin A-inducible fragile site FRA8E was mapped to 8q24.1 in which various loci implicated in genomic instability are located. This region is involved in various chromosomal rearrangements associated with genetic diseases and cancers. Furthermore, this region was shown to be the most common integration site of human papillomavirus, especially HPV16 and HPV18 in cervix cancers. In the present study aimed at positional cloning of the FRA8E locus and integration site of HPV16 DNA sequence (Fig.1), we have constructed a YAC contig at 8q24.1 region by using published integrated physical mapping data and by two-color fluorescence in situ hybridization (FISH) method. To identify YAC clones covering the FRA8E region,FISH analysis was carried out using chromosome spreads prepared from a heterozygous carrier of FRA8E. We identified a YAC clone which produced split signals at the expressed site of fra(8)(q24.1). This YAC clone was found to contain both FRA8E locus and breakpoints involved in chromosomal rearrangements associated with Langer-Giedion syndrome (LGS) and hereditary multiple exostoses (EXT1). Using P1 phage clones, the FRA8E locus was further localized to a genomic region (ca. 300kb) of the EXT1 gene (data not shown). The integration and amplification site of HPV16 DNA sequence in cervic cancers was shown to be involved in an extensively broad region including c-myc oncogene, but it did not coincide with the FRA8E locus. The identifications of YAC clones and P1 phage clones presented here will facilitate the cloning and characterization of DNA sequences associated with FRA8E locus and HPV16 DNA integration site. Fig.1. An integrated physical map of YAC clones at 8q24.1, and locations of the FRA8E locus and HPV16 DNA-integrated site.

[Publications]

3. 5 BIO-MEDICAL SCIENCES Genetics

3. 5. 11 Construction of Physical Map Covering the Maijor AT Locus

Masatake Yamauchi, Takashi Imai, Toshiyuki Saito, Tada-aki Hori, Satsuki Tsuji, and Tetsuya Saeki

Keywords: genomic instability, ataxia telangiectasia, radiosensitive, genome analysis

Ataxia telangiectasia (AT) is a human genetic disease that is genetically recessive and manifests itself as cerebellar ataxia and dilation of blood vessels. AT patients also show immunodeficiency, genomic instability, and high incidence of tumors, as well as hypersensitivity to ionizing radiations. To investigate the function of the gene product which defect is responsible for AT phenotypes, we covered the entire region of the major AT locus at 11q22-24, where the localization of the responsible gene(s) was suggested by genetic linkage analyses, using yeast artificial chromosome (YAC) clones. Cosmid contig covering the AT locus was constructed using YAC clones, and the cosmid clones were used to search for the DNA regions that can be transcribed. Four independent transcripts were identified by using the CpG island rescue method.To know the exact positions and directions of the transcribable units (＝genomic genes), we constructed a restriction map of the overlapping cosmid clones covering the major AT locus (Fig.1). Three genes, ATM, NPAT, and ACAT, were mapped within ～ 300 kb of the genomic DNA, although the exact position of another gene, T451, has not been determined on the cosmid contig yet. ATM gene is possibly a candidate gene that is responsible for AT phenotype, since mutations were identified in ATM gene of AT patients. The functional basis of ATM gene has not been established. Genomic DNA fragments covering ATM gene were extremely unstable during proliferation in the cosmid vector, and a bacterial artificial chromosome (BAC) clone was employed to cover the region. NPAT gene was mapped very close to ATM gene. The first exons of the two genes, ATM and NPAT were ～ 500 bp apart and orientation of the two genes was in a head- to-head manner. This suggested that ATM and NPAT share the same promotor sequence, and that their transcription were possibly regulated by the same factors. This implies that NPAT can be another possible candidate gene for AT phenotype. ACAT was originally reported as a genomic gene coding for mitochondrial acetoacetyl-coenzyme A thiolase by another group, and mapped at the junction of 11q22.3 and 11q23.1 by us using FISH method. ACAT gene was found to be in a intron of NPAT gene. The restriction map of the cosmid contig constructed here will provide useful informations for further analyese of the genes identified, including gene transfer experiments for functional complementation of the AT phenotypes using each of genomic genes as well as mutation analyses of AT patients and heterozygotes. Fig.1 YAC(yeast artificial chromosome, represented by solid bars) contig was constructed to cover the entire genomic region between S1818 and S1819, wher the existence of the responsible gene(s) for the radio-sensitive AT (ataxia telangiectasia) phenotype was suggested by the genetic linkage analysis. The small open circles on solid bars of a YAC clone represent STS markers detected. Cosmid contig (gray bars) was then constructed by using human genomic DNA prepared from the YAC clones. BAC (bacterial artificial chromosome, shaded bar) clone was also employed to cover the region where cosmid clones were highly unstable. Two genes, ATM and NPAT, were newly identified in the contig region by using the CpG island rescue procedure, and their positions and transcriptional-directions

were determined (represented by arrows). Another gene, ACAT (gray box), was also identified and mapped in as intron of NPAT gene. It has already been reported by another research group as a genomic gene coding mitochondrial acetoacetyl-coenzyme A thiolase.

[Publications]

3. 5 BIO-MEDICAL SCIENCES Genetics

3. 5. 12 Molecular Analysis of the Relationship between Radiologically Induced Mutations and Genes Involved in the DNA Precursor Metabolism

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

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

Despite recent achievements in analyses of the primary structure of the human genome, its functional aspect has yet to be analyzed. Our three year project aims to examine the possibility of 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, and mutations, and primary structures of the genes involved in the pathway.

The metabolic pathway of the DNA precursor compounds is one of the most strictly regulated pathways in all organisms, including humans. Defects in the key regulatory enzymes in the pathway result in nucleotide pool imbalances that eventually lead to occurrence of mutations in genes. Genetic variations in the DPM pathway among the human population may possibly play an important role in the development of various diseases including neoplastic transformation induced by ionizing radiation and chemical compounds in the environment. The existence of genetic variation in the DPM pathway among human population is evident, being supported by the population who tend to manifest gout. Gout is a disease that is often caused by genetic defects in the DNA precursor metabolism. The number of gout patients in the Japanese population is estimated to be nearly one million, although the proportion having genetic gout is not known.

To investigate the relationship between the DNA precursor metabolism and the mutagenesis caused by the envirobnmental mutagens at the molecular level, we have started to isolate genes involved in the DNA precursor metabolism of various organisms, human, mouse, and fission yeast. Fission yeast is thought to provide an ideal system to investigate the function of the mammalian genes isolated, since the procedures for homologous recombination to disrupt the target gene and its function are established.

We isolated seven human cDNA clones and nine yeast cDNA ones in the first budget year. In the second budget year, we isolated eight more human cDNA clones, three rodent ones, and three yeast ones. Their nucleotide sequences were determined in parallel with isolation, completely for a full-length cDNA clone of the human purH gene, and partially for other incomplete-length ones. Human purH cDNA was composed of 2068 nucleotides, and a single open reading frame encoding 592 amino acids was identified. Biological function of the human purH gene was confirmed by functional complementation achieved by introducing the cDNA clone in the expression vector to the mouse purH-negative mutant cells.

[Publications]

Yamauchi, M., Seki, N., Mita, K., Saito, T., Tsuji, S., Hongo, E., Morimyo, M., Shiomi, T., Koyama, H., Ayusawa, D., and Hori, T.,: DNA Research, 2:269-275,19

3. 5 BIO-MEDICAL SCIENCES Genetics

3. 5. 13 dentification of Chromosomal Localization of FRA16q22.1

Hideo Tsuji, Ei-ichi Takahashi 1, Motoi Murata 2 and Kazuei Mita (1 Ohtsuka Pharmaceutical Co.; 2 Chiba Cancer Center Res. Inst.)

Keywords: fragile site, FRA16B, YAC, FISH

Some of the fragile sites on human chromosomes were recently isolated, which showed the involvement of (CGG) n repeat expansion and the association of genetic diseases and chromosomal instability. The heritable fragile site FRA16q22.1 (FRA16B) has been suspected as being predisposed to the chromosome 16 inversion found in patients with acute myeloid leukemia M4Eo, because some of the patients expressed the FRA16B in normal peripheral lymphocytes. We examined the involvement of FRA16B with the breakpoint of chromosome 16 inversion by fluorescence in situ hybridization (FISH) using the genomic clones as a probe, which contained the CBFB gene located in the breakpoint. FISH analysis with chromosomes expressing FRA16B revealed that the CBFB gene was located distal to the fragile site, indicating the dissociation of the FRA16B to the cancer breakpoint. The isolation of genomic DNA including FRA16B is needed to clarify the genomic structure and the biological significance of FRA16B. To identify the chromosomal localization of FRA16B, yeast artificial chromosome (YAC) clones containing human genomic DNA located in 16q22.1 were isolated and chromosomal localization was examined by FISH. The DNA of the clone 821G9 carrying the STS D16S3238 and D16S3021, and the clone 768F12 with the STS D16S3021 and D16S186 crossed the FRA16B. The clones possessing the STS D16S3238 had the FISH signal proximal to the fragile site, while the location of the clones containing D16S2629 was distal. The detailed analysis of the STS content data clarified the order of the STS, D16S3238, D16S3021, D16S186, and D16S2629 from the centromere to the telomere. Thus the of FRA16B was placed between D16S3021 and D16S186.

[Publications]

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．14 ERCC8 Involved in Cockayne Syndrome Group A Is Mapped to Human Chromosome 5p12-p13.1

Tadahiro Shiomi, Naoko Shiomi, Yoshinobu Harada, Manabu Koike, Ei-ichi Takahashi 1 ,Toshiki Ito 2 and Masaru Yamaizumi 2 (1 Otsuka Pharmaceutical Co.; 2 Kumamoto Univ.)

Keywords : ERCC8, group A Cockayne syndrome, irradiation hybrid, complementation test, gene mapping

Cells from patients suffering from xeroderma pigmentosum (XP) or Cockayne syndrome (CS) are hypersensitive to UV- irradiation and are known to be defective in some steps of the nucleotide excision repair. Using a cell fusion technique, excision repair-defective XP cells have been divided into seven genetic complementation groups (A to G), whereas in CS two complementation groups (A and B) have been identified. In addition to these human mutant cells, large numbers of UV-sensitive repair deficient mutants have been isolated from established rodent (Chinese hamster or mouse) cells as another source of UV- sensitive mutants and classified into at least 11 genetic complementation groups.

To assign a human gene complementing the defect of the group 8 rodent mutant (ERCC8), the mutant (6L1030) cells were fused with the UV-resistant hybrid (6L1030 X human fibroblast) cells irradiated with X-rays, followed by four cycles of UV- selection (irradiation hybrid formation). To obtain the human sequences which are retained in the fourth irradiation hybrid, 6LH4R1, a lambda phage library was constructed with genomic DNA of 6LH4R1 and screened with total human DNA as a probe. Five independent phage clones (L6LH4R-1-5) which showed positive signals were isolated. The lengths of human DNAs containing in these clones ranged from 13 kb to 20 kb. A direct mapping, which is based on FISH combined with replicated prometa-phase

R-bands, was carried out, with DNAs of the lamda phage clones as probes. We examined 50 typical R-banded prometa phase chromosome for each probe. The fluorescent signals for each probe were observed on the short arm of chromosome 5 at p12-

13.1 band, strongly suggesting that ERCC8 gene is assigned to the same band of chromosome 5.

Cell fusion complementation tests measuring unscheduled DNA synthesis (UDS) as a cellular marker are often difficult with UV- sensitive rodent cell lines, because a high proportion of cells are in the S phase of the cell cycle, which hampers discrimination of grains due to UDS from those due to regular DNA synthesis. To overcome this difficulty, we adopted recovery of RNA synthesis (RRS) instead of UDS as a marker for complementation tests, since RRS after UV irradiation in 6L1030 cells is severely depressed. Among the nine excision repair defective complementation groups in humans (seven groups for XP and two groups for CS), only three genes corresponding to XP group E, XP group F, and CS group A cells have not been identified. Since the characteristics of XP group E cells are quite different from those of 6L1030 cells, cell fusion was performed with XP group F and CS group A cells.

These two cells also have impaired RRS after UV irradiation. Since the fused cells were placed between 6L1030 cells and reference cells on the same coverslip in the complementaion tests, minor changes in

grain numbers of the fused cells were recognized easily by comparison with the grain numbers of the two parental cells plated on each side of the fused cells. An increased RRS was observed in heterokaryons of 6L1030 cells with XP group F (Nps1) or CS group B (CS1MO), but not with CS group A (Mps1) . To confirm the fidelity of the inspection method, we counted the grains of the fused cells. Although the grain number in UV-irradiated mononuclear 6L1030 cells varied in different experiments, possibly due to differences in culture conditions, the grain number in heterokaryons was higher than in the parental cells in all combinations except 6L1030 cells with CS group A (CS2SE, Mps1) cells. These results indicate that 6L1030 cells belong to CS group A.

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3．5 BIO-MEDICAL SCIENCES Genetics

3．5．15 Generation of Mouse Strain with Knockout Mutations in the DNA Excision Repair Gene (xpg)

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

Keywords: xpg, mouse model, gene targeting, group G xeroderma pigmentosum, excision repair

Xeroderma pigmentosum (XP) is a rare inheritable disorder which is characterized by a clinical and cellular hypersensitivity to the ultraviolet (UV) component of the sunlight spectrum. In eight complementation groups of XPs, XP-G is one of the most rare and phenotypically heterogeneous groups. Although the XPG gene that complements defects of the XP-G cells has been shown to function as a DNA endonuclease, it was suggested to have an additional function essential for viability. To investigate further unknown functions of the XPG gene, mice with non-functional xpg (the mouse counterpart of the human XPG gene) alleles were generated using gene targeting methods and embryonic stem cell technology.

To generate a mutant allele of xpg, the pMER5／TV2 targeting vector was designed to generate an insertional mutation in one exon of the mouse xpg gene. Targeted ES cells were injected into C57BL／6 blastocysts to generate chimeric mice which transmitted the mutant allele to F1 offspring. The heterozygous F1 mice were interbred in an attempt to obtain mutant homozygous mice. Of 163 mice born, 35 (21.5%) were severely runted and died between 0 and 23 days post partum. DNAs were successfully collected from 31 of 35 dead mice. Southern blot and PCR analyses of these DNAs revealed that all 31 mice were homozygous for the targeted xpg gene. The 128 survivors were either wild-type (39, 23.9%) or heterozygotes (89, 54.6%).

Although the size of the mutant homozygotes at birth seemed to be almost the same as those of wild-type and heterozygous mice, growth of the xpg mutant homozygotes was severely inhibited thereafter. Fig.1 shows a photograph of three littermates with different genotypes at 16 days old, demonstrating the severe inhibition of growth associated with xpg deficiency.

To examine the effects of the insertional mutation on expression of the xpg gene, total RNA from newborn mice was analyzed by Northern blotting. No stable (intact or truncated) xpg transcript was detected in the homozygous mice using the xpg cDNA as a probe. In heterozygous mice, the xpg mRNA content was approximately half of that in wild-type mice. These findings indicate that disruption of the xpg gene by our method was effective. Primary fibroblasts were isolated from newborn mice of all three xpg genotypes and their susceptibilities to UV were examined by measuring colony forming ability after exposure to various doses of UV. The fibroblasts from xpg mutant homozygotes proliferated in culture as rapidly as in wild-type cells, and plating efficiencies were almost the same among these fibroblasts. The fibroblasts from mutant homozygous mice were hypersensitive to UV (254 nm) irradiation. Cells from heterozygotes were as resistant to UV irradiation as wild-type cells. The UV survival curve for mutant homozygous fibroblasts was almost the same as that for cells from a severe XP-G patient.

Fig.1. Three littermates with severe growth inhibition associated with xpg deficiency.

[Publications]

3．5 BIO-MEDICAL SCIENCES Genetics

3．5．16 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

Manabu Koike, Yoichi Matsuda, Tsuneyo Mimori＊, Yosh-nobu Harada, Naoko Shiomi and Tadahiro Shiomi (＊Keio Univ.)

Keywords: Ku p70, Ku p80／XRCC5, repair gene, Northern analysis, gene mapping

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. In yeast, double-strand break repair occurs mainly by homologous recombination. In mammalian cells, however, double-strand break repair seems to occur by a different pathway. In the case of rodent cells, ionizing-radiation sensitive mutants have been isolated and divided into at least eight X-ray repair cross complementation (XRCC) groups. Out of these, XRCC4, XRCC5, and XRCC7 mutants have been demonstrated to be defective in the double-strand break repair systems. Recently, the XRCC7 gene has been shown to be defective in scid mouse and to encode the p350 protein.

The DNA-dependent protein kinase (DNA-PK) complex is a nuclear serine／threonine protein kinase composed of a catalytic subunit p350 (DNA-PKcs) and a DNA binding component named Ku. The Ku autoimmune antigen, abundant in the nuclei and capable of binding to DNA, is a complex composed of two subunits of 70 and 80 kDa, which are designated as Ku p70 and Ku p80, respectively. The antigen is recognized by autoantibodies in sera of certain patients with systemic lupus erythematosus, Graves disease and scleroderma-polymyositis overlap syndrome. The Ku p70 and Ku p80／XRCC5 genes are involved in DNA double-strand break repair and V(D)J recombination, and their gene products are the components of the DNA-PK.We have determined chromosomal location of the mouse Ku p70 and Ku p80 ／XRCC5 genes by both in situ hybridization and molecular linkage analysis: 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. Molecular linkage analysis using interspecific backcross mice revealed that the murine Ku p70 locus was localized at 0.7 cM terminal to D15Mit1, and that the murine Ku p80／ XRCC5 locus was at 0.7 cM proximal to D1Mit46 . These results suggest that these genes originate from a common genetic linkage in mammalian evolution. To determine 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.

[Publications]

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3．6 BIO-MEDICAL SCIENCES Radiotoxicology

3．6．1 Effects of CBMIDA and Zn-DTPA in Drinking Water on Removal of Plutonium in Rat

Satoshi Fukuda, Haruzo Iida, Yuyuan Hseih← and Wenzhi Chen← (←Shanghai Institute of Materia Medica)

Keywords: oral administration, CBMIDA, plutonium, Zn-DTPA

｛The effects of the oral administration of CBMIDA [catechol-3,6- bis(methyleiminodiacetic acid)], given in drinking water, on the removal of plutonium in rats were compared to those of Zn-DTPA. Male Wistar rats received a daily dose of 120 米 mol▼kg or 1,200 米 mol▼kg of CBMIDA or Zn-DTPA, given in drinking water, for 4 weeks after plutonium injection. The content of plutonium in bone, liver, kidney, spleen, and testis was measured by liquid scintillation spectrometry after treatment of the organs by a wet ash method. The plutonium content of each organ in the 120 米 mol▼kg groups of CBMIDA and Zn-DTPA was not significantly reduced compared to the control. The content of plutonium in the 1,200 米 mol▼kg groups of both CBMIDA and Zn-DTPA was, however, significantly reduced, to 65.7⊙ (p～0.001) and 76.8 ⊙ (p～0.05), respectively of the control in bone, and to 66.6 ⊙ (p ～ 0.05) and 44.0 ⊙ (p ～ 0.01), respectively, in liver. There were no significant decreases in kidney, spleen, and testis in the 1,200 米 mol▼kg dose CBMIDA group, but there were significant decrease in these organs in the 1,200米mol▼kg dose Zn-DTPA group. These results showed that, when given orally, CBMIDA was more than effective in removing plutonium from bone than Zn-DTPA, a finding similar to the results achieved with the intravenous injection of both agents in our previous study.

[Publications]

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3．6 BIO-MEDICAL SCIENCES Radiotoxicology

3．6．2 Correlation between Cell Death and Induction of Non-rejoining Chromatin Breaks by X-rays and Carbon-Ion Beams

Masao Suzuki, Yoko Kase, Tatsuaki Kanai and Koichi Ando

Keywords: non-rejoining chromatin break, PCC, carbon-ion beams, LET

{ We reported that the induction rate of the initially measured chromatin breaks per cell per Gy, which were obtained by counting the number of chromatin fragmentations detected by the premature chromosome condensation (PCC) technique, on normal human fibroblast cells was almost constant, but that the number of remaining breaks after 12h of post-irradiation incubation differed with the LET between 0.3keV▼ 米 m and 230keV▼ 米 m for 137Cs gamma-rays and carbon-ion beams. This LET dependence of non-rejoining chromatin breaks was the same as the LET dependence of cell death.

{ In order to clarify the relationship between the radiosensitivity and the induction of non-rejoining chromatin breaks by qualitatively different types of radiation on different radiosensitive human cell lines, we examined the correlation between the cell death and the induction of non-rejoining chromatin breaks on normal human cells and human tumor cell lines by using X-rays and carbon-ion beams accelerated by the HIMAC.

{ The RBE values of cell death by carbon-ion beams relative to X-rays were 1.1 to

1.4 for 13 keV▼米m beams and 2.5 to 2.9 for 77 keV▼米m beams. The induction rate of non-rejoining chromatin breaks per cell per Gy was the highest for 77 keV▼

米 m beams on all of the cell lines. The results concerning the relationship between cell death and non-rejoining chromatin breaks indicated that the cell line which was more sensitive to cell death had higher induction of non-rejoining chromatin breaks. These results suggest that there was a good correlation between the cell death and the induction of non-rejoining chromatin breaks on various cell lines.

{ We have now begun to examine use of the PCC technique for predictive assay in cancer radiotherapy.

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Advances in Space Research, 18, (1▼2)127-(1▼2)136, 1996.

4. CLINICAL RESEARCH

4．1 Dose-volume Histogram Analysis of High Dose Rate Intracavitary Brachytherapy for Uterine Cervix Cancer

Atsuro Terahara, Takashi Nakano, Atsuko Ishikawa, Shinroku Morita, Hirohiko Tsujii, and Yuzuru Nakamura

Keywords: uterine cervix cancer, dose-volume histogram, brachytherapy

We retrospectively analyzed the relationship between dose distribution and local control using a dose-volume histogram (DVH) in patients with cancer of the uterine cervix treated by definitive radiotherapy including intracavitary brachytherapy.Twenty-five patients with squamous cell carcinoma of the uterine cervix who underwent definitive radiotherapy between August 1987 and April 1994 were selected for the present study. They included 15 patients with local control and 10 patients with local recurrence. In principle, these patients were treated with 50 Gy of external beam pelvic radiotherapy and a point A dose of 24 Gy, in four fractions, of intracavitary brachytherapy. The DVHs of tumor volumes were calculated by superimposing three-dimensional dose distributions on computed tomography (CT) images taken before brachytherapy (Fig. 1).Differential DVHs revealed a tendency for the portion of the total tumor volume to which the delivered dose was low to be larger in patients with local recurrence. The tumor volumes and the absolute dose volumes of which the absorbed dose was less than 24 Gy [DV (＜ 24 Gy)] were significantly larger in patients with local recurrence than those in local control patients (p = 0.02 and 0.03, respectively). The actuarial local control rate of patients whose absolute DV (＜ 24 Gy) was more than 50 ■ was significantly lower than that of patients with absolute DV (＜ 24 Gy) of 50 ■ or less (p = 0.02). The percent DV (＜ 24 Gy) was not significantly different in the two groups. In patients with larger tumor volume, the absolute DV (＜ 24 Gy) was also larger and a strong linear correlation was noted between them. The analysis of dose distribution of brachytherapy using DVH was useful to evaluate the quality of dose distribution quantitatively. The absolute dose volume was considered more important than the percent dose volume for evaluation of the clinical outcome. Our study suggested that unfavorable dose distribution for the tumor volume in brachytherapy was one of the reasons for poor local control in patients with large tumor volume.

[Publications]

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Fig.1 Differential DVH by local control (absolute volume). (a) Absolute version of differential DVH of patients with local control (n＝15). Most patients in this group had a small tumor volume for which irradiation dose was relatively low.(b) Absolute version of differential DVH of patients with local recurrence (n＝10). There was a trend that the portions of the tumors receiving lower dose were relatively large compared with (a).

4. CLINICAL RESEARCH

4．2　Correlation Between c-erbB-2 Oncogene and Cell Proliferation Parameters in Radiation Therapy for Cervical Cancer.

Takashi Nakano,Kuniyuki Oka,Atsuko Ishikawa, and Shinroku Morita.

Keywords : CerbB2, Ki-67, cervical cancer, radiation therapy, growth fraction

Background. Although c-erbB-2 oncoprotein expression (CerbB-OPE) is regarded as being associated with tumor cell proliferation and prognosis, the correlation between CerbB-OPE and cell proliferation parameters has not been fully analyzed. Methods.

Immunohistochemical study was performed on 64 cervical cancer patients treated with radiation therapy. Prognosis was analyzed by CerbB-OPE, growth fraction determined with Ki-67 immunohistochemistry (Ki-GF) and the mitotic index of proliferating cell population (pMI). Results. CerbB-OPE was observed on the cell membrane of cancer cells. Positivity of CerbB-OPE, which was 42.4% in total,increased significantly with stage progression. No significant differences were observed between histologic subtypes. 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 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 CarbB(■) ones($P<0.01$). The difference in survival was mainly due to local recurrence rather than distant metastasis. There were significant correlations between prognosis and Ki■GF and pMI. Conclusions. The poor prognosis of the cervical cancer with CerbB■OPE was due to local recurrence following radiation therapy. The correlations between CerbB■OPE and Ki■GF and pMI suggest that c■erbB■2 oncoprotein may play an important role in cell proliferation status of cancer of the uterine cervix.

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Nakano, T., Oka, K., and Taniguch, Y.: Cancer Res., 56, 2771■2775, 1996.

Oka, K. Nakano, T., and Hoshi, T.: Cancer, 77, 2280■2285, 1996

4. CLINICAL RESEARCH

4．3 Mn■SOD Expression Correlates with p53 Status and Local Recurrence of Cervical Carcinoma Treated with Radiation Therapy

Takashi Nakano, Kuniyuki Oka, Atsuko Ishikawa, Shinroku Morita and Hirohiko Tsujii

Keywords: Mn■SOD, p53, Radiation therapy, Cervical cancer, prognosis

Background. Manganese superoxide dismutase (Mn■SOD) inactivates the radiation effect by removal of the radiation induced toxic superoxide radicals. The purpose of this study is to assess the correlation between Mn■SOD, radiation sensitivity and prognosis following radiation therapy. Methods. The Mn■SOD, p53 protein, and c■erbB2 oncoprotein expressions in 52 specimens from patients with cervical cancer treated with radiation therapy were investigated immunohistochemically. The frozen sections were stained using anti human Mn■SOD, and anti p53 monoclonal antibodies, and anti c■erbB■2 oncoprotein polyclonal antibody, following avidine biotine peroxidase complex methods. Correlations between the Mn■SOD expression and prognosis or failure patterns were analyzed. Additionally, correlations between p53 and c■erbB■2 oncoproteins and Mn■SOD expression were investigated. Results. Positive expression of Mn■SOD in cervical cancer was 48.1%. No significant difference in positivity of Mn■SOD expression was noted according to stage and histologic subtypes. The 5■year survival rate of Mn■SOD positive patients was 42.5%, significantly poorer than the 77.0% of Mn■SOD negative patients ($p<0.05$). Fig.1 Analyzing the failure patterns, patients with Mn■SOD expression showed a significantly higher incidence of local recurrence than those without. However, there was no difference in distant matastasis between them. Although both p53 and c■erbB■2 oncoprotein expressions were significantly associated with the prognosis of the same patients, Mn■SOD expression was associated with p53 oncoprotein expression but was not with that of c■erbB■2 oncoprotein. Conclusions. Our results demonstrate that the Mn■SOD level of cancer cells is correlated with local control and is an important prognostic factor in radiation therapy for cervical cancer.

Mn■SOD level may help explain the intrinsic radiation sensitivity of cervical cancer cells.

[Publications]

1)Nakano, T., Oka, K, and Taniguch, Y.: Cancer Res., 56. 2771■2775, 1996.

2)Oka, K., Nakano, T., and Hoshi, T.: Cancer, 77, 2280■2285, 1996.

5.ENVIRONMENTAL SCIENCE

5．1　Reevaluation of the Biological Half-Time of Cesium in Japanese Male Adults

Masafumi Uchiyama

Keywords: cesium, biological half-time, male, adult, Japanese

In a previous study in the 1960's, an average cesium biological half-time of 86 days with 1 SD of 21 days was reported for 23 selected Japanese male adults. It was determined under a quasi-equilibrium condition by combining data from whole-body counting and urinalysis for 137Cs resulting from atmospheric nuclear weapon tests. Recently increased 137Cs body burdens were observed in returnees from the former USSR territories due to the Chernobyl accident. Measuring their body burdens repeatedly with an appropriate interval, the retention followed a linear line when 137Cs in the body was expressed as a logarithm. Biological half-times were obtained for 22 Japanese male adults in this way. An average of 100 days with 1 SD of 32 days was observed.

When two individuals with extremely high half-times were excluded, an average of 91 days with 1 SD of 24 days was estimated. After a 20 year elapse in time, the difference in the biological half-time of cesium at a confidential level of 90 % was not significant but had an increasing tendency. When the two Japanese groups that were measured in the 1960's and the 1980's were combined , the biological half-time of cesium was 93±27 days for 45 subjects (Fig.1) and 88±23 days for 43 subjects, without the 2 extremely large values, respectively. Insignificant differences in the Cs biological half-time between the 1960's and 1980's were indicated in Sweden, Germany and Russia for male adults. The reference biological half-time, thus, should be 90 days for Japanese male adults. Cesium biological half-time for individual subjects might be subject to both differences and changes in physique and body constituents. Four subjects took part in both the studies in the 1960's and the 1980's. In the 1960's the four subjects were in their twenties. Their changes in biological half-time were various. One subject had lost 20 % of his potassium but his body weight in the 1980's was similar to his 1960's weight. His biological half-time in the 1980' s was also similar to his value in the 1960's. Three other subjects had biological half-times in the 1980's that were 30 to 80 % longer than during the 1960's.

One subject among them had lost 14 % of his potassium. The two other subjects had similar potassium contents for both times even though their body weight had increased by about 10 kg. This analysis suggests that the biological half-time of cesium increases with time despite the natural decrease in the potassium pool that occurs with aging.

[Publications]

Fig.1 Distribution of biological half-times of cesium in 45 Japanese male adults.

5.ENVIRONMENTAL SCIENCE

5．2　Three Decades' Study on 137Cs Body Burden and Its Consequent Internal Dose for Japanese Male Adults

Masafumi Uchiyama, Takeshi Iinuma, Tomio Ishihara, Tetsuo Ishikawa, Masaki Matsumoto

Keywords : body burden, 137Cs, Japanese, male adults, internal dose

The present study analyzing 137Cs body burden was commenced at the National Institute of Radiological Sciences (NIRS) in 1964. In the previous year, 1963, fallout from nuclear weapon tests was at its peak. The subjects measured were a group of Japanese adult male scientists, mainly from NIRS. The youngest were in their twenties and the oldest in their fifties. Periodical measurements of 137Cs body burden using a whole-body counter were performed. The man-year of the subjects measured ranged from 30 to 219. In the early period from 1963 to 1972, a whole-body counter with 2 sets of plastic detectors was usually used to measure the body burdens for 15 minutes in a stationary mode. For about 20 years, from 1972 onwards, a whole-body counter with two 8-in diameter by 4-in thick NaI(Tl) detectors was used. Measurements were carried out in a scanning mode of 5 cm per minute. To calibrate the measurements a block phantom of an adult Japanese male with an average physique for the early 1960's was filled with a known amount of 137Cs. The internal dose estimated in the present study was the total body dose. This was because external methodology needed to measure the organ weight in order to determine the effective dose had not and as yet, has not, been developed. The MIRD method was applied to estimate the internal dose from accumulated 137Cs after the absorbed fractions were modified according to the total body weight of each individual subject.Average body burdens of 137Cs decreased linearly from a maximum of 520 Bq with one standard deviation (SD) of 43 Bq in 1964 to 132 Bq with 1 SD of 26 in 1967. Nuclear weapon tests by China decelerated this decreasing tendency by a factor of 1.9. From 1974 to 1985, 137Cs body burdens kept a constant level of around 20 Bq. The Chernobyl Accident brought about a small increase in the body burden. In 1987 there was a peak of 56 Bq. The consequences of this increase were detected for 5 years.Internal dose due to 137Cs body burden changed with time as indicated in Fig. 33. The annual dose attained a maximum of 20μSv in 1964. The committed dose of 82μsv was estimated for internally deposited 137Cs resulting from unclear explosions in 1961 and 1962. The consequence from the Chernobyl accident to the dose of the present group was estimated to be 5.6μSv. The health effect of the dose on the group was evaluated to be insignificant. Before 1964, the increase in 137Cs body burden was estimated by both urinalysis and the deposition of fallout due to atmospheric nuclear weapon tests by the USA and USSR in the 1950's. Urinalysis data indicated that the body burden rose and fell symmetrically before and after 1964. Thus the internal dose from 137Cs could be estimated to be roughly 170 μSv in average for the whole period for the group measured.

[Publications]

Fig.1 Three decades of changes in the annual averages of the internal dose due to 137Cs to an adult male group in Japan.

5.ENVIRONMENTAL SCIENCE

5．3　Intestinal Absorption of 14C-chitosan in Rats

Yoshikazu Nishimura, Yoshito Watanabe, Jia Ming Hong＊, Hiroshi Takeda andMasae Yukawa (＊ Institute of Nuclear Agricultural Sciences, China National Nuclear Corporation)

Keywords: 14C-chitosan, intestinal absorption, distribution

Chitosan is an insoluble polysaccharide consisting of β-(1,4)-linked N-acetylglucosamine(GlcNAc) units, and is abundant in the marine environment. This polysaccharaide, which is mainly derived from crustaceans, such as crabs and shrimps, has recently attracted attention as a useful biomass. Our previous research has proved that chitosan can reduce the bioavailability of radiostrontium in rats. However, the reduction mechanism and basic biokinetics are not clear yet. The purpose of this study was to investigate the absorption and the basic biokinetics of chitosan in rats. Carbon-14 labeled chitosan [(C8H13NO5)n] was synthesized by Tokai Research Laboratories and purchased from Dai-ich Chemical Company. It's radiochemical purity was assessed using High Performance Liquid Chromatography (HPLC) and was 98.7 %. 14C-chitosan was administered orally to rats. It was observed that the radioactive material was distributed quite extensively in their tissues. The relative concentration (radioactivity per gram of wet tissue／radioactivity administered per gram of body weight) of radioactivity in the tissues, increased sharply for the first few hours and then decreased smoothly. From the sixth to the twelfth hour after administration, the relative concentration in most of the rat tissues increased, except in the intestine where it decreased. During the sixth to the twelfth hour, the sum of the total amount of radioactivity in the tissues except for the intestine was about 5 % of the originally administered dose of 14C-chitosan. These results indicate that 14C-chitosan was probably absorbed from the gastrointestinal tract, and was metabolized and excreted quickly without re-bioavailability. The radioactivity in the serum, in extracts from the liver and in the contents of small intestine were separated using Gel Permeation Chromatography and measured for 14C. A great peak was found in high molecular weight region compared with the standard 14C-chitosan. This sharp peak had a retention volume the same as Bovine Serum albumin (Fig. 34). The sharp peak could be made to disappear if the proteins contained in the serum or liver were removed. In addition, a third peak was found in all 3 samples, regardless of whether the protein was removed or not. It followed the other peaks and was eluted with lower molecular weight range retention volume products, the same as chito- oligosaccharides. These results suggest that some 14C-chitosan is possibly absorbed into the blood, liver and other tissues, either directly or after being degraded to small molecular compounds.

[Publications]

Fig.1 Analysis of serum and liver by GPC.

5. ENVIRONMENTAL SCIENCE

5．4　Biokinetics and Dose Estimation of Radiocarbon in Rat

Hiroshi Takeda, Shoichi Fuma and Tetsuo Iwakura

Keywords: radiocarbon, 14C-compounds, biokinetics, dose estimation, rat

Radiocarbon (14C) is formed as a by-product of nuclear power generation, and a part of it is released to the environment. Although the estimated releases of 14C from presently operating nuclear power facilities are still quite small in comparison with natural and other man-made sources, the introduction of new nuclear technologies, coupled with reprocessing of spent nuclear fuel elements and the disposal of radioactive wastes, are virtually certain to raise the quantity of 14C that is produced and potentially released. In recent years, concern about its potential long-term hazard to man as a source of radiation exposure has prompted a number of assessments of proposed nuclear fuel cycles and facilities. 14C is often released into the environment in the forms of carbon dioxide or bicarbonate. A part of such inorganic 14C is considered to be transformed into organic 14C by photosynthsis in plants during food chain transfer. Human exposure to 14C is, therefore, occurs by intake of air and water containing inorganic 14C and by intake of food containing organic 14C. The purpose of the present study is to estimate relative radiotoxicity of different chemical forms of 14C, which will be taken by ingestion of water or food. Sodium bicarbonate as inorganic 14C, and amino acids (leucine and lysine), fatty acids (palmitic acid and oleic acid), glucose and thymidine as organic 14C, were administered to rats by a single ingestion. After the ingestion, the rats were killed at various time intervals and dissected to obtain various tissue or organ samples. These samples were combusted in an oxidizer which automatically adds an aquatic scintillator and the radioactivities in the combustion water were determined with a liquid scintillation counter. The data were expressed in terms of relative concentration, defined as the percentage of radioactivity administered per g of body weight of individual rat. The time-course of relative concentrations of 14C in the rat tissues after the ingestion of various 14C-compounds was compared. It was found that there were differences in the biokinetics among the ingested 14C-compounds. Lower incorporation and retention of 14C was observed after ingestion of 14C-bicarbonate, followed by 14C-thymidine ingestion. As shown in our previous study using ^{3}H-thymidine, the major part of ingested thymidine is decomposed rapidly in the gastrointestinal tract. This should explain the lower incorporation and retention of 14C-thymidine. Higher retention of 14C was observed after ingestion of 14C-amino acids and 14C-fatty acids. Based on these biokinetics data, radiation dose was calculated for individual tissues of rats due to ingestion of the same amount of radioactivity per g of body weight (Table 1). Tese results indicated that radiation doses depended upon the chemical form when 14C was ingested. Significant differences were observed between inorganic 14C and organic 14C, except for 14C-thymidine. The highest radiation doses to the majority of tissues were observed when 14C-amino acids were ingested. The doses from 14C-leucine and 14C-lysine were, respectively, 15-34 and 29-70 times higher than the doses from 14C-bicarbonate. The results of the present study indicated that 14C incorporated into protein through the food chain should be considered as an important source term of human exposure to environmental 14C.

5.ENVIRONMENTAL SCIENCE

5．5 Application of a River Runoff Model Designed for the Kanto Plain to the Nuclear Site, Rokkasho Village

Kiriko Tanaka-Miyamoto, Yoshikazu Inoue, Tetsuo Iwakura and Takashi Iyogi

Keywords:

Kanto Plain in 1954-1995 was compared with the observed values of river waters in Rokkasho Village in 1991-1995. Fig.1 compares three response curves with the observed data of river waters in Rokkasho Village. The basic response curve (solid line) shows TSn for the Kanto Plain calculated using precipitation data in the Kanto Plain. The annual mean value of tritium concentration for precipitaion in Rokkasho Village for 1994-95 was 1.5 times as much as that for the Kanto Plain during the period of this study. The next response curve (dashed line) is based on the input data multiplied by this factor of 1.5 times for the period from 1954-1995. This curve only fits the observed data for river water whose tritium concentration is the lowest in this period. To obtain a better fitting, the size of the second layer reservoir of groundwater in the Rokkasho Village was changed to "one third" of that in the Kanto Plain. The final response curve (cross-point line) shows a better fit to the observed data for river water whose tritium concentration is highest in this period. This two-stepped modification demonstrated that the river runoff model developed for the Kanto Plain is applicable to other local areas including Rokkasho Village. There are several reasons why the amount of fallout tritium deposition by precipitation in Rokkasho Village was 1.5 times higher than that in the Kanto Plain in 1994-95. It is known that on a global scale there is gradient in the latitudinal distribution of tritium content in the atmosphere. The higher the latitude, the higher the tritium content, and this is especially evident in the northern hemisphere. Recently it was reported that the Japanese Islands are strongly influenced by atmospheric currents from the Asian Continent which contain greater levels of fallout tritium. This continental air mass has a greater influence on the northern part of the Japanese Islands than the southern. Also, the areas under study vary in their geological scale. Water volume of the second layer in the Rokkasho Village was estimated to be one third of that in the Kanto Plain. Thus the volume of the second layer of groundwater is about twice that of annual precipitation in Rokkasho Village.

[Publications]

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Fig.1 Tritium concentration in river waters observed in Rokkasho Village and comparison with the response curve TSn. The same symbols mean samples from the sa

5.ENVIRONMENTAL SCIENCE

5．6 Influence of Microbial Activity on Technetium Behaviour in Soil under Waterlogged Condition

Keiko Tagami and Shigeo Uchida

Keywords : technetium, immobilization, redox potential, soil, waterlogging, microbial activity

The behaviour of Tc in soil depends upon its chemical forms. Technetium is known to exist in all valence states from +7 to -1. The most stable form of Tc in natural aqueous solutions in equilibrium with the atmosphere is the pertechnetate form, TcO_4^-. Therefore, under aerobic conditions, Tc is present as TcO_4^-, which has a high geochemical mobility and bioavailability. However, the form changes through a combination of factors such as redox conditions and microbial activity in soils. The anaerobic condition can be supplied in waterlogged rice paddy soil, which is common to Japan and other Southeast Asian countries. Since rice is main food in these countries, it is important to predict 99Tc behaviour in rice paddy field. In this study, we have carried out radiotracer experiments to obtain the influence of microbial activity on 99Tc behaviour in soil under the anaerobic (waterlogged) condition by measuring Eh values. The microbial activity is expected to be controlled by addition of glucose (0.5w% of soil) and the activity causes a change in the soil redox condition. Air-dried soil and sterile soil samples with and without glucose (A-0, A-0.5, S-0 and S-0.5) were used to clarify the role of microorganisms in the Tc transformation. The soil samples, 40 g amounts of each, were placed in polystyrene vessels (120 mL) and the depths of the soil in the vessels were 4 - 4.5 cm. They were carefully waterlogged with 60 mL of 99Tc solution (JRIA, 370 Bq／mL). The vessels were sealed with parafilm to prevent entry by microorganisms from the air. At each sampling time, the surface solution was collected and its Eh value was measured. The results for Eh and the relative concentrations (RC: C／C0) of 99Tc in the surface soil solution of AD-0, AD-0.5, S-0 and S-

0.5 as a function of time are shown in Fig. 1. The RC was defined as "the activity in a solution at each sampling time (C)" divided by "the initial activity in the solution (C0)". The pH values of all soil samples were almost constant during the period. The RC of 99Tc in the surface solution of AD-0.5 decreased over time and at the end of the experiment, it was almost zero, however, those of AD-0, S-0 and S-0.5 were decreased about 20 to 40% by the end of the experiment. We found that Tc in the soil solution was adsorbed on the soil under the low Eh condition. It is, however, difficult to explain whether microbial activity affects only generation of low Eh or transformation of Tc in soil. From these results, it is clear, however, that the low Eh condition was generated not only by waterlogging, but also by microbial activity.

[Publications]

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Fig.1 Relative concentration of 99Tc and Eh in the surface solutions of AD■0, AD■0.5, S■0 and S■0.5 as a function of time.

5.ENVIRONMENTAL SCIENCE

5．7　Determination of Major and Trace Elements in Mushrooms, Plants and Soils Collected from Japanese Forests

Satoshi Yoshida and Yasuyuki Muramatsu

Keywords : ICP-MS, trace elements, mushroom, plant, soil, forest

Measurements of major and trace elements in biological and soil samples within a forest are needed to expand our knowledge of the elemental composition of the forest ecosystem and to predict migrations and effects of chemical elements. Most research efforts concerning the elemental migrations in forest ecosystems have concentrated on the major nutrient elements. Therefore, the distribution and transfer of many trace elements are still unknown. In this study, inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) were used to measure many major and trace elements in plant, mushroom and soil samples collected in Japanese forests. Sample preparation and analytical conditions were investigated to set up a simple routine procedure for measuring a large range of elements. Fifty elements were determined for soil samples. For plant and mushroom samples, 25 elements were determined. Concentrations of some trace elements such as Zn, Pb, Cd, Bi, Sn and Sb in forest soils tended to be the highest in the surface soil layer, indicating the importance of atmospheric deposition on the total contents in the soils of these elements. In comparison with the element contents of plants, the mushroom contents could be characterized by low Mg, Ca, Sr and Ba amounts. Transfer factors (TFs) were estimated from the ratio of "concentration in plant or mushroom on dry weight basis" to "concentration in the surface soil on dry weight basis" (see Fig. 1). The TFs of Co, Ba, lanthanide elements, Th and U were very low in all plant and mushroom samples. Mushrooms tended to accumulate Cu, Zn, Rb, Cd and Cs. The TFs of Cs for mushrooms were one or two orders higher than those for other plants growing in the same forest. High concentrations of radiocesium discharged through nuclear weapons testing and nuclear accidents have been reported in many countries. The high TFs for stable Cs obtained in this study indicated that mushrooms are important Cs accumulators and radiocesium is taken up from soils together with stable Cs.

Multi-element capability of ICP-MS can provide information on the distribution of many trace elements in forest ecosystems. In addition, analyses of both plants and soils provide in situ TFs. Species specific accumulation and plant-availability of the elements can be estimated by the TFs.

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Fig.1 Transfer factors for plants and mushrooms collected from a pine forest in Tokai, Ibaraki.

5.ENVIRONMENTAL SCIENCE

5．8　Dietary 232Th and 238U Intakes for Japanese and Contributions of Imported Foods to Internal Doses

Kunio Shiraishi and Masayoshi Yamamoto

Keywords :

Furthermore, an attempt was made to calculate the effects of imported foods on internal dose by using the present analytical results. Foodstuffs of 174 kinds were purchased from markets in Mito city, Ibaraki Prefecture. Statistical consumption data of 1987 were used for collection of the food samples. Standard Reference Material (SRM) 1571 Orchard Leaves was also obtained from the National Institute of Standards and Technology, and used for quality control. The foodstuffs purchased, approximately 74■, were divided initially into 30 food groups and dry■ashed in a muffle furnace at a final temperature of 400℃. The ash obtained was collected, according to four final groups, as mixed ash samples. The four groups were as follows: group 1＝eggs, milk, and milk products; group 2＝bean, animal, and fish products; group 3＝ fruits, vegetables, and potatoes; and group 4＝ cereals, oil, and others. Statistical consumption data were also collected according to these four groups. An aliquot of the mixed ash samples was taken and completely decomposed with a mixture of concentrated nitric acid and perchloric acid. The sample solution was analyzed by inductively■coupled plasma mass spectrometry (ICP■MS). Daily intakes of 232Th in the four respective food groups were found to be 0.047, 0.526, 1.05, and 0.599mBq per person. For 238U, intakes of groups 1, 2, 3, and 4 were 0.088, 2.96, 11.8, and 0.60 mBq, respectively. Total 232Th and 238U intakes were estimated to be 2.22 mBq and 15.5 mBq per person per day, respectively.　Using the present results, preliminary calculation was conducted. Calculation results are shown in Table 7. Six food groups were used: 1)milk products, 2) meat products, 3) fish products, 4) leafy vegetables, 5) roots and fruits, and 6) grain products, in accordance with the report of United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR 1993). First, one assumption must be made that all foodstuffs analyzed in the present work were self■supplied in Japan (i.e. all foodstuffs taken were not imported ones). Next, suitable values obtained from the present results in the four food groups were arbitrarily assigned into the six food groups. Annual intakes of each food group (shown in the 2nd column in the Table) were referred to the data of the National Nutritional Survey. Self■supply ratios of foods in Japan of 1990 (shown in the 3rd column), were used. Importation ratios were one minus the self■supply ratios. The radioactivities of imported food groups (shown in the 6th column) were referred to UNSCEAR data. The activities were reported as averages collected from data of the northern hemisphere for a mild climate region. Radioactivities from self■supplied food were obtained by multiplying the amounts of annual intakes (■／y), self■supply ratios, and radioactivities (mBq／■) of／each food group self■supplied in Japan. The share of the imported foods was calculated in the same way. In the case of 232Th, the annual intakes of self■supplied and imported foods were 500 mBq and 556 mBq per person, respectively. Total annual intake was 1056 mBq／y. If all foods were self■supplied in Japan, (i.e. ratios of self■supply are all one), total annual intake would be 804 mBq. Owing to the ratio of B／A (about 1.3), a 30% increment

would be obtained as the contribution of imported foods. If ratios of self■supply were all 0.5 or all 0.3, the ratios of B／A would be higher, 1.8 or 2.1, respectively. Dietary intake of 238U would be about 1.6 times higher due to the importation of foodstuffs from foreign countries, as obtained by the same calculation method. Effects of imported food on internal exposures were estimated using the present analytical results and UNSCEAR data. But, in order to get more refined effects, radioactivities in many food groups and／or in each supplying country, must be clarified. After collecting these data, a good computer code should be established for the dose calculation concerning the effects by food importation.

[Publications]

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5. ENVIRONMENTAL SCIENCE

5．9 239＋240Pu and 137Cs Distributions in Seawater from the Japan Sea

Masatoshi Yamada, Tatsuo Aono, Shigeki Hirano

Keywords: Japan Sea, 239＋240Pu , 137Cs, activity ratio, inventory

Artificial long-lived radionuclides have been spread worldwide through nuclear weapons tests making it necessary to understand the degree of artificial radioactive contamination in the marine environment. The purpose of our investigation was to determine concentrations of the artificial radionuclides, 239＋240Pu and 137Cs, in seawater from the Japan Sea, and to discuss the behaviour of 239＋240Pu by measuring distributions of 239＋240Pu／ 137Cs activity ratios and 239＋240Pu inventories in the water column. Seawater samples were collected at the Yamato Basin and at the Tsushima Basin of the Japan Sea during the N93- 02 cruise of the R／V "Natsushima", Japan Marine Science and Technology Center. 239＋240Pu and 137Cs are mainly delivered to the ocean by fallout derived from atmospheric nuclear weapons tests. The 239＋240Pu／ 137Cs activity ratios can be used to evaluate the effect of scavenging on the 239＋240Pu by comparing deviations from the global fallout ratio. The 239＋240Pu／ 137Cs activity ratio versus water depth is plotted in Fig.1. The dotted line represents 239＋249Pu／ 137Cs global fallout ratio of 0.023 corrected for 137Cs decay to 1993. At the Tsushima Basin station, the 239＋240Pu／ 137Cs ratio increases gradually with depth from 0.005 at a 100 m depth to 0.035 at a 1400 m depth. The ratio in the upper 750 m depth is less than the global fallout value of 0.023. At the Yamato Basin station, the ratio has a minimum layer at a 500 m depth and increases largely with depth to 0.12 at a 1400 m depth. This ratio of 0.12 is five times that of the global fallout ratio. These results support the proposal that scavenging and removal of 239＋240Pu relative to 137Cs take place in the upper layer of the Japan Sea.The 239＋240Pu inventories at the Yamato Basin station are estimated to be 12.5, 31.4, and 42.7 Bq／■ over the depth interval 0-500m, 500- 1400m, and 1400m- bottom, respectively. The inventories at the Tsushima Basin station are almost the same. The 239＋240Pu inventories at the Yamato Basin station are 14.5, 36.2, and 49.3 % of those in the whole water column over the depth interval 0- 500 m, 500- 1400 m, and 1400 m - bottom, respectively. Half of the 239＋240Pu is present in the deep water column over the depth interval 1400 m - bottom; this fact indicates that 239＋240Pu relative to 137Cs is transported downward rapidly. The 239＋240Pu inventories in the whole water column are estimated to be 86.6 and 85.2 Bq／■ at Yamato and Tsushima Basin stations, respectively. These values are about two times greater than that of the estimated direct fallout input at the same latitudes of 30- 40° N. The 239＋240Pu inventory in the water column is about 90 % of that in the total (water column＋sediment column).

These results suggest that a large amount of 239＋240Pu delivered to the Japan Sea still remains in the water column.

[Publications]

Fig1. The 239＋240Pu／ 137Cs activity ratio versus depth at Stn. 1 in the Yamato Basin (circles) and at Stn. 2 in the Tsushima Basin (squares).

6.APPENDIX

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List of Keywords

(-)-anisomycin

(S)-pyroglutamic acid γ-rays

137Cs

14C-chitosan 14C-compounds

2, 6-diaminopurine resistance 239＋240Pu

8q24.1

activity ratio

adenine-phosphoribosyltransferase adult

antibiotic antibiotics

ataxia telangiectasia ATM

ATM gene

ATM transcripts auto activation beam spill

biokinetics biological half-life biological half-time

biological membrane body burden Bombyx

bone damage

bone histomorphometry brachytherapy

brain evoked potentia bromodeoxyuridine

calorie restriction CAR bacillus

carbon-ion beams cartilage

casein CBMIDA CD8＋

cDNA catalog CerbB2 cervical cancer

major histocompatibility complex male

male adults malformations mammalian cells mammary gland medaka

megakaryocyte progenitor cells(CFU-Meg) metsbolism

mice

microbial activity microsatellite locus mitotic recombination Mn-SOD

molecular dynamics Monte Carlo simulation mouse

mouse brain mouse model

mRNA secondary structure mRNA stability

mushroom mutagenesis mutant induction mutation site myeloid cells

N-methylformamide neoantigen specific antibody neonatal period

neuronal migration neutrons

nitric oxide

non-rejoining chromatin break Northern analysis

NPAT

nucleotide sequence nude mice

optical reflection oral administration ospetopenia osteoporosis

cesium chiral ligand

chlorine dioxide chromatin structure

chromosomal fragile site chromosome 11q22-23 chromosome duplication cis-dihydroxylation common mode

comparative genome mapping complememtation test complement Cls

copper(II) complex cumene hydroperoxide Dahl-Iwai salt-sensitive rat development discontinuous translation distribution dithiocarbamate

DNA

DNA damage

DNA dependent PK

DNA double-strand break dose estimation

dose-response relationship dose-volume histogram dosimetry intercomparison

drug metabolizing enzyme system early response gene

electron cyclotron resonance embryo transfer environmental model

ERCC8 ESR

evolutionarily equivalent excision repair

EXT1 gene extrasensory Fe plot

FISH

fish

fission yeast forest

oxidation

P 2 peak latency p53

parathyroid-thyroid PCC

Penning PET

phylogenetic relationship planar lipid bilayer

plant plutonium polarity effect

polyhydroxylated pyrrolidine polyubiquitin gene

pre T cell

prenatal chronic irradiation prognosis

programed cell death protein kinase C proton beam

purine metabolism qi-gong

quantitative Northern hybridization radiation carcinogenesis

radiation damage radiation protection radiation therapy

radiation-induced T Cell lymphomas radical scavenger

radiocarbon radiosensitive radiosensitive mutants random rough surface rat

rat hepatocytes RBE

recovered dose redox potential rem-counter repair enzyme repair gene retrotransposon RFLP

FRA16B FRA8E

fractionated irradiation fractionated radiation fragile site fragmentation

free radical free radicals gamma rays

gamma-ray irradiation gene mapping

gene structure gene targeting genetic defects genetic variation genome analysis genomic instability germfree mouse globin groundwater

group A Cockayne syndrome group G xeroderma pigmentosum growth fraction

GVHD

GVL effect harmonic content heavy ions hematopoiesis

hepatocellular tumors hepatoma

high LET radiation histomorphometry Hprt

hydrological cycle hydroxyl radical

hypertophic chondrocytes ICP-MS

immobilization immunohistochemistry in situ hybridization information transfer ingestion

intakes

ripple

Rokkasho nuclear site Rp II

RT-PCR

ryanodine receptor saturation

SCE

SCID mouse sense shielding shielding SHRSP

signal transduction skin reaction

soil

spin trapping strain difference subconsciousness suggestion sulfamerazine surgery

synchrotron power supply T cells

T4 endonuclease V (T4 endo V) technetium

tert-butyl hydroperoxide thermoluminescent dosemeter thorium

thymic lymphoma thymine dimer Ti 3 +

tohate

trace elements transcriptional regulation translation elongation factor tritium

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