(Annual Report) 1994-1995

1. physics

NIES

2. chemistry

NIES

1. Synthesis of Polyhydroxylated Pyrrolidines from (S)-Pyroglutaminol and (R)-Serine

Nobuo Ikota

Key words: polyhydroxylated pyrrolidine, (S)-pyroglutaminol, (R)-serine, allylic strain, rutenium tetraoxide,glycosidase inhibitor.

Polyhydroxylated pyrrolidines are potent inhibitors of glycosidases andmannosidases and have therapeutic utility in the treatment of various diseases suchas viral infection. In connection with our studies on the synthsisi of polyhydroxylatedamines, we describe here the synthesis of (2R,3R,4S)- and (2R,3R,4R)-3,4-dihydroxy-2-hydroxymethylpyrrolidine (1 and 2) and (2R,3S,4R,5R)- 3,4-dihydroxy-2,5-dihydroxymethylpyrrolidine (3) from (S)-pyroglutaminol and (R)-serine. cis-Dihydroxylation of a,b-unsaturated lactam (4) prepared from (S)-pyroglutaminol, gave a diol (5) in 82% yield, which was further converted to(2R,3R,4S)-3,4-dihydroxy-2-hydroxymethyl-pyrrolidine (1) by reduction of thelactam carbonyl with borane-tetrahydrofuran complex followed by removal of theprotecting groups. On the other hand, the steric course of dihydroxylation of Z-olefin(6)derived from (R)-serine might be different from the case of the a,b-unsaturatedlactam (4) due to the allylic strain. Koskinen reported that cis-dihydroxylation of Z-olefin (7) with a catalytic amount of OsO4 gave a diol (8) selectively. The Z-olefin(6a) was prepared from N-tert-butoxycarbonyl-O-trityl-(R)-serine methyl ester in65% yield and reduction of of 6a with diisobutylaluminum hydride followed by tert-butyldimethylsilylation afforded 6b in 75% yield. In contrast with the result byKoskinen, cis-dihydroxylation of 6a and 6b with OsO4 (0.15 eq.) in the presence ofN-methy- morphine N-oxide in acetone-H2O gave 9a and 9b predominantly (9a:10a=2.2:1; 9b:10b=4.2:1). Protection of the diol (9b) with is opropylidenegroup followed by removal of TBS group with tetrabutylammonium fluoride affordedthe alcohol (9c) in 79% yield. Mesylation of 9c followed by cyclization withpotassium tert-butoxide in tetrahydrofuran (THF) furnished the pyrrolidine (11),which was hydrolyzed with 10% aqueous HCl-MeOH at 60–C to give thehydrochloride of (2R,3R-4R)-3,4-dihydroxy-2-hydroxymethylpyrrolidine (2) in 71%1. yield. (2R,3S,4R,5R)-3,4-dihydroxy-2,5-dihydroxymethylpyrrolidine (3) wassynthesized from 9a. After protection of 9a with isopropylidene group and removalof methyl ester with aqueous NaOH, 9d was converted into the N,O-dimethylhydroxyamine amide, which was reacted with vinyl magnesium bromide inTHF to afford the enone (12) in 45% yield. Reduction of the enone (12) with NaBH4in the presence of CeCl3 in EtOH gave an allylic alcohol (13) as a single isomer.Mesylation of 13 followed by cyclization with potassium tert-butoxide gave 5-vinylpyrrolidine (14a) in 74% yield. This cyclization may proceed via the allyliccation derived from mesylate to afford the pyrrolidine 14a, which could bethermodynamically stable isomer. Ozonolysis of 14a followed by reductive workupwith NaBH4 gave the alcohol, which might be useful intermediate for the synthesisof 2, 7a-diepialexine. 14b was further converted into the hydrochloride of(2R,3S,4R,5R)-3,4-dihydroxy-2,5-dihydroxymethyl-pyrrolidine (3) with acidichydrolysis in 72% yield.

Publications:

(1) Ueda, J., Ikota, N., Hanaki, A. and Ozawa, T. : Biochem. Mol. Biol. Int., 33, 1041-1048, 1994.

(2) Ikota, N. : Heterocycles, 41, 983-994, 1995.

(3) Hanaki, A., Saito, M. and Ikota, N. : Nippon Kagaku Kaishi, 388-393, 1995.

(4) Hanaki, A., Nagai, A. and Ikota, N. : Chem. Lett., 611-612, 1995.

(5) Tomioka, K., Kanai, M. and Ikota, N. : Heterocycles, in press.

3.1 BIO-MEDICAL SCIENCE Biochemistry and Biophysics

1.DNA Binding and Compaction Activity of Human Histone H1

Masahiko Takahagi, Ikuko Furuno, Kouichi Tatsumi

Keywords: DNA binding, DNA compaction activity, histone H1, junction DNA

In the course of search for DNA processing factors in human cells, we have found an activity that preferentially binds to branched DNA , whose structure includes four-way and three-way junctions, and loop-out type DNAs. Through affinity chromatography steps, the activity was purified to homogeneity as a single band detected in SDS-polyacrylamide gel electrophoresis. From biochemical properties and partial amino acid sequences, it was identified with human histone H1 protein.

Several experiments have demonstrated that H1 is the abundant protein that localized in nucleosome linker sites on chromosome. Although it has been proposed that H1 acts for folding of nucleosome fiber and repression of general transcription, the true function remains unclear. Recent studies also displayed that the protein is not essential in levels of chromosome condensation, nuclear assembly, and even existence for Tetrahymena cells. These facts seem to imply that H1 plays a role in unusual metabolism to change chromatin structures like DNA repair. Alternatively, since there is the possibility that DNA binding of H1 to various DNAs could compete with that of other factors toward common target, the specificity may be regulated by some mechanisms.

For right understanding of the function of H1, its recognition and reactivity to defined DNA structures were further analyzed with gel shift assay. As a result, we found that H1 has an activity to compact the spatial occupancy of DNA, whose structure must contain both multiple junction-like sites and DNA flexibility or peculiar configuration, such as single-strand circular DNA. The compaction form of H1-DNA complex was also confirmed by electron microscopic observation.

Our finding shows that H1 directly works the alteration of DNA structure under a situation where is satisfied rigid requirements for substrate, and provides a clue to know about early stage of DNA metabolisms.

2. Free radical scavenging ability of liver cytosol in radiosensitive SCID mouse

Osami Yukawa, Tetsuo Nakajima, Toshihiko Ozawa, Yoshie Shimazu and Junichi Ueda

Keywords : SCID mouse, radiosensitivity, radical scavenging ability, antioxidative enzymes

It has been well known that SCID mouse is highly sensitive to ionizing radiation. On the other hand, it was established that active oxygens or free radicals produced by radiation are the main cause to induce a wide variety of radiation effects on living organisms, and intracellular radical scavenging ability is thought to be implicated to the radiation sensitivity. In the present study, therefore, radical scavenging ability and activities of antioxidative enzymes in liver cytosol were compared between SCID mouse and its wild type C.B.17 which has the same genetic background as SCID mouse, for determining a mechanism of the radiosensitivity. When radical scavenging ability in liver cytosol was measured as the trapping capacity of 1,1-diphenyl-2-picrylhydrazyl(DPPH) which is a stable free radical, more than 20% of the ability was lower in SCID than that of the wild type, suggesting that the protection against active oxygens or free radicals is attenuated in SCID mouse liver.

Further studies to clarify the cause of this low level of radical scavenging ability were carried out by determining activities of antioxidative enzymes. GSH-peroxidase, GSH-reductase and superoxide dismutase showed low activities in SCID mouse liver cytosol as compared with those in the wild type. Therefore, it was concluded that low radical scavenging ability in SCID mouse was, at least a part, resulted from the low activities of antioxidative enzymes. Changes in other intracellular antioxidative substances are now under analyzing.

High sensitivity of SCID mouse to ionizing radiation is presumed to be resulted from its immune deficiency. However, low ability to scavenge free radicals was also shown in SCID mouse liver in the present study. This result suggests that low radical scavenging ability in tissues is also implicated to high sensitivity of SCID mouse, though it is not clear whether or not there is some direct relationship between immune deficiency and low radical scavenging ability.

3. Involvement of lipid peroxidation in radiation- induced translocation of protein kinase C in cultured rat hepatocytes.

Tetsuo Nakajima and Osami Yukawa

Keywords: rat hepatocytes, signal transduation, protein kinase C, lipid peroxidation, membrane damages

We have already demonstrated that active oxygens produced by radiation induce lipid peroxidation and simultaneous changes in the process of Ca2+ signaling system (IP3 induction, IP3-mediated Ca2+ release etc.)in cultured rat hepatocytes. We have also shown that radiation activates protein kinase C(PKC), which participates in cellular signal transduction pathways, and assumed that the activation is due to translocation of PKC from cytosol to membranes in the cells. In this study, further investigation was carried out on the radiation- induced activation of PKC using lower dose of radiation than the dose in the previous experiments and direct evidence was obtained for radiation-induced translocation of PKC molecules . The activity of PKC was increased in the membrane fraction and decreased in the cytosolic fraction after g-irradiation of hepatocytes both with 5Gy and 50Gy. The effect of the activation of PKC was found to be in a dose dependent manner. Changes in the intracellular distribution of PKC after irradiation was determined by the method of binding assay of PKC with [3H]PdBu(phorbol-12,13-dibutyrates). PdBu bound was decreased in the cytosols and increased in the membrane after irradiation. These results indicate that the radiation-induced activation of PKC in rat hepatocytes is due to translocation of the enzyme molecules from cytosol to membranes. Furthermore, we examined effects of radical scavengers(thiourea, trolox) on the translocation of PKC. Treatment of the cells with thiourea didn't significantly alter the radiation- induced increase in PKC in the membrane fraction of the irradiated rat hepatocytes . However, trolox, a water-soluble analogue of vitamin E, inhibited the increase of PKC in the membrane fraction. We have also observed that trolox had a better effect than thiourea on the inhibition of radiation-induced lipid peroxidation in rat hepatocytes.

These results suggest that the radiation -induced translocation of PKC is mediated by lipid peroxidation of hepatocyte membranes.

4.Detection of a Point Mutation in ErbB2 in the DNA of the Radiation-induced Mammary Tumors.

Keiko Suzuki and Hiroshi Inano

c-ErbB2 is an oncogene which encodes a cell surface membrane protein, and its product is a receptor of a growth factor. In the human mammary cancers and variously induced mammary tumors of rodents, mutations of the oncogene erbB2 have often been reported. Neuro/glioblastoma is frequently induced in the offspring of the rat which has been administered ethylnitrosourea on the day 15 of the pregnancy. The point mutations, from T to A, were detected in erbB2 in the DNA of the neuro/glioblastomas derived from individual rats. The mutation resulted in the substitution of valine with glutamic acid in the transmembrane domain of the product.

We irradiated rats with -rays of 2.6 Gy on day 21 of lactation, and then the rats were implanted with a pellet of a synthetic estrogen, diethylstilbestrol, for one year. Mammary tumors were detected in 96.4% of the rats. DNA was prepared from a normal lactating mammary gland and 14 radiation-induced mammary carcinomas. The DNA's were analyzed by PCR-SSCP method to detect a point mutation in erbB2 corresponding to the transmembrane domain of the receptor protein. The primer DNA oligonucleotides were synthesized to amplify erbB2 sequence of genomic DNA corresponding to the transmembrane domain. The 32P-labeled DNA fragments which were amplified by PCR from the DNA's of the normal lactating mammary glands and the mammary tumors were separated by polyacrylamide gel electrophoresis after denaturation at 80xC for 3 minutes and immediate cooling. The gel was dried on a filter paper, and the DNA fragments were detected by autoradiography. As a result the DNA fragments derived from the mammary tumors represented the identical mobilities with the fragments amplified from the DNA of the normal lactating mammary glands. Thus, any point mutations were not detected in erbB2 sequence corresponding to the transmembrane domain in The DNA of the radiation-indnced mammary tnmors.

5. Chemoprevention by Dietary Dehydroepiandrosterone (DHEA) against Promotion Phase of Radiation-induced Mammary Tumorigenesis in Rats

Hiroshi Inano, Hiroko Ishii, Keiko Suzuki, Hiroshi Yamanouchi, and Makoto Onoda

Keywords: Mammary tumor, ~-rays, Dehydroepiandrosterone, Chemoprevention, Promotion phase

DHEA is a major secretory steroid of the adrenal glands and its biological significance is known to be as a precursor steroid in the biosynthesis of androgens and estrogens. It has been reported that low plasma level of DHEA may be associated with an increased risk of breast cancer in women. The present study was designed to evaluate the anti-carcinogenic activity of DHEA against diethylstilbestrol(DES)-dependent promotion of radiation-induced mammary tumors. When pregnant Wistar-MS rats received whole body irradiation with 2.6 Gy ~-rays at day 20 of pregnancy, and were then implanted with a DES pellet for an experimental period of 1 year under feeding of a control diet, a high incidence (96.2%) of mammary tumors was observed. Administration of dietary 0.6% DHEA together with DES implantation significantly decreased the incidence (35.0%) of mammary tumors (Table). For clarification of the mechanism of the chemopreventive action by DHEA, we measured hormone levels in serum of DHEA-fed rats. In the DHEA diet rats, the concentration of estradiol exceeded, by approximately 6-fold, that in the control rats, while the levels of progesterone and prolactin were decreased by 30 and 45%, respectively. Interestingly, DHEA feeding prevented DES-induced hypertrophy of pituitary glands and DES-induced high level of prolactin in pituitary glands detected by immunohistochemical studies, but stimulated the development of mammary glands more than that in control rats treated with DES alone by whole mount observation. These findings suggest that DHEA has a potent preventive activity against the promotion phase of radiation- induced mammary tumorigenesis. The observed anti-carcinogenicity of DHEA is specific to some action of the steroid. DHEA is metabolized mainly to androst-5-ene-3~,17~-diol by 17~- hydroxysteroid dehydrogenase in the mammary glands in rats. Since DHEA itself has an extremely low relative binding affinity for estrogen receptor, binding of the metabolite to estrogen receptor is well known. Androst-5-ene-3~,17~-diol derived from dietary DHEA competes with DES for high-affinity intracellular binding sites for estrogen, thus reducing the promotion activity of DES. Also, DHEA feeding results in peroxisomal proliferation in the liver. The induction of peroxisomal enzymes leads to an increase of oxidative stress by overproduction of H2O2 during activation of ~-oxidation of fatty acids. H2O2 may inactivate HMG-CoA reductase which catalyzes a rate limiting reaction for biosynthesis of farnesyl-pyrophosphate before its degradation by catalase in the cells. Subsequently, inactivation of HMG-CoA reductase may cause suppression of isoprenylation of p21ras, which is a critical step in the cell-transforming activity of oncogenic ras proteins. Therefore, inhibition of posttranslational processing of p21ras by DHEA may contribute to its chemopreventive effects.

In conclusion, these findings indicate a protection effect of DHEA against the DES-dependent promotion

of radiation-induced mammary tumors in rats by a multi-function chemopreventive mechanism.

[Publications]

(1) Suzuki, K., Ishii, H., Yamanouchi, H., Wakabayashi, K.,**Takahashi, M. and Inano, H.:** Intl. J. Cancer, 56, 413-417,1994.

(2) Yamanouchi, H., Ishii, H., Suzuki, K., Onoda, M., **Wakabayashi, K. and Inano, H.:** Intl. J. Cancer, 60, 230-234,1995.

(3) Inano, H., Ishii, H., Suzuki, K., Yamanouchi, H., Onoda, M.**and Wakabayashi, K.:** J. Steroid Biochem. Mol. Biol. 54, 47-53, 1995.

1.Studies of Cell Killing and Induction of DNA Double-Strand Breaks by Active Oxygen Species in Mouse Mutant Cell Line Deficient in DNA Double-Strand Break Repair

Masahiro Murakami, Kiyomi Eguchi-Kasai and Koki Sato

Keywords: DNA double-strand break repair, active oxygen, mouse mutant cell line

It is said that ionizing radiation can induce cell death, mutation and cell transformation. These events appear to be related to hydroxyl radicals produced during the radiolysis of water. Although the other active oxygen species such as hydrogen peroxide (H2O2) and superoxide also generate ·OH radicals, the actual mechanisms by which these products exert biological effects differ markedly.

Hydrogen peroxide has been suggested to cause cell-killing by inducing DNA double-strand breaks. But, because a cellls ability to cope with active oxygen species appears to depend on many factors, it is not entirely clear how these processes are influenced by the cell's DNA repair capacity. We have used a mouse cell line deficient in DNA double-strand breaks repair (SL3-147) to clarify the role that double-strand break repair has in ameliorating the adverse biological effects induced by various active oxygen species.

This mutant cell line (SL3-147) shows different sensitivities to X-rays, hydrogen peroxide, paraquat and menadione when compared to the parental cell line (LTA). The respective D0 values of LTA and SL3-147 were 1.5 and 0.4 Gy in the case of acute dose X-irradiation. SL3-147 was more sensitive to fractionated dose of X-rays (total dose 1-5Gy, 1Gy/day), hydrogen peroxide (ゥ0.86mg/ml) and paraquat (100-500mg/ml), but was less sensitive to menadione (1-6mg/ml) in side by side comparisons to LTA cells. The induction of DNA double-strand breaks caused by active oxygen species on these cell lines were analyzed by pulsed-field gel electrophoresis. DNA double-strand breaks were induced by H2O2 and menadione in both LTA and SL3-147, whereas those induced by paraquat were found only in SL3-147. The greater number of DNA double-strand breaks in SL3-147 appears to account for its greater sensitivity to X-rays and paraquat. DNA damage other than double-strand breaks or injury to non-DNA targets, however, are responsible for the differences between LTA and SL3-147 in their sensitivities to hydrogen peroxide and menadione.

Publication:

1) Murakami, M., Eguchi-Kasai, K. and Sato, K.: Mutat. Res., 336, 215-221, 1995.

2. The Human Gene Encoding the Largest Subunit of RNA Polymerase II

Kazuei Mita, Hideo Tsuji, Mitsuoki Morimyo, Ei-ichi Takahashi*, Mitsuru Nenoi, Sachiko Ichimura, Masatake Yamauchi, Etsuko Hongo and Akiko Hayashi

(*Otsuka Phamaceutical Co.)

Keywords: Nucleotide sequencing, RpII, gene structure

In the cource of a complementary study of a Chinese hamster CHO-K1 cell tsTM4 mutant with human HeLa genomic DNA, we have isolated the gene for human RNA polymerase II large subunit (RpII LS) and determined its genomic organization with nt sequencing. Determination of structure of RpIILS is essential to know whether the whole gene is needed or a special part of the gene is required to compensate the defect of the mutant. To complete the gene structure of human RpIILS, several genomic clones overlapping each other were isolated from a HeLa genomic library and sequenced. Fig. shows the genomic organization of human RpIILS gene that consists of 29 exons spanning around 32 kb. The deduced amino acid sequence is identical with that derived from cDNA by Wintzerith et al. although several silent differences in nucleotide are found. The gene structure of the mouse RpIILS gene reported by Ahearn et al. is shown in Fig. together with that of human for comparison. The CDS presented high nucleotide conservation of 90% between human and mouse. The 5′ untranslated region (UTR) also showed high conservation (84%). The 3′ UTR showed 62% identity between the two species, being less conserved than the nucleotide sequences in the other regions. However, the vicinity of a polyadenylation signal gave high conservation. On the other hand, large differences in length and sequence were observed in introns between human and mouse. Homology search showed that the big difference came from the insertion of interspersed repetitive sequences, Alu in human and B1 in mouse. We could deduce the consensus sequence of splice junctions in human RpIILS gene, which was completely identical with that derived from GenBank database search. The sequence of the 5′ flanking region is highly conserved as compared with that of the mouse RpIILS and contains several SP1-binding sites, a CCAAT sequence and a sequence homologous to a heat-shock element. In addition, several inverted repeats and palindrome sequences were involved in the 5′ upstream region. Those suggest that the 5′ flanking domain of RpIILS would be highly structured which may be responsible for transcriptional regulation.

Publication:

1) Mita, K., Tsuji, H., Morimyo, M., Takahashi, E., Nenoi, M., Ichimura, S., Yamauchi, M., Hongo, E. and Hayashi, A.: Gene, 159, 285-286, 1995.

3. Expression of the Bombyx mori b-Tubulin Encoding Gene in Testis

Kazuei Mita, Mitsuru Nenoi, Mitsuoki Morimyo, Hideo Tsuji, Sachiko Ichimura, Masaji Sawai* and Koei Hamana(*Takara Shuzo Co. Ltd., **Gunma University)**

Keywords: DNA sequencing, cDNA clone, Northern analysis

It is now clear that several isoforms of b-tubulin (Tub) are encoded in the multigenes expressed developmentally or tissue-specifically in several species. We have isolated a Tub cDNA from the cDNA library of Bombyx mori (Bm) testes in the late 5th instar and pupal stages and determined its nucleotide sequence. This would be a first clone for Bm Tub gene, judging from the search in the most recent databases. Its amino acid sequence, deduced from the nucleotide sequence, is highly homologous to the major vertebrate Tub (80-82% homology). Comparison of the amino acid sequence of Bm Tub with those of Drosophila melanogaster (Dm) isoforms (the ubiquitous b1, the testis-specific b2 and a developmentally regulated b3) gives 81.2% homology with b2, 79.8% with b1 and 77.4% with b3, respectively. Sullivan and Cleveland classified five isoforms of chicken Tub. Comparison of the amino acid sequence of the Bm Tub with those five chicken Tub isoforms shows that the Bm Tub amino acid sequence is most conserved in chicken testis-specific b3 among five isoforms. The comparative study of the amino acid sequences of Tub of vertebrate isoforms, Drosophila isoforms and Bm reveals that the C-terminal domain enriched in acidic amino acids is highly divergent, whereas the amino acid sequences of structured domains are highly conserved.

Total RNAs of testes or other tissues ofBm larva or pupa were extracted by the LiCl/urea procedure. Isolation of poly(A)+RNA was carried out by selective binding to oligo(dT)-latex. Northern analyses revealed that the Bm Tub gene was expressed only in testis (Fig. 1a). Moreover, the transcription of the gene was developmentally regulated as shown in Fig. 1b. The nucleotide sequence of the probe for the Tub gene used in Fig. 1 is the complementary sequence of the 3l-coding sequence, which corresponds to the C-terminal variable region. The mRNA was observed at a high level in pupal stage, while it was kept at a low level up to the stage just before pupation. The morphological study reported that appearance of spermatozoa begins the day before the sponning stage of the larvae and the number of spermatozoa continuously increases until the 9th day after pupation. These observations suggest that the Bm Tub gene product would function mainly in the formation of mature motile spermatozoa. Kemphues et al. studied the Dm testis-specific Tub synthesis during spermatogenesis and reported that the synthesis of Dm Tub in the Dm sperm axoneme was not detected until the time of puparium formation, while the Tub was mainly synthesized after pupation. The expression pattern of Dm testis-specific b2-Tub gene seems to be quite similar to that of the Bm Tub gene. Low level of mRNA observed in 5th instar larval testes (Fig. 1b) may suggest that the protein product of the gene also functions in meiotic spindle, which was proved in Dm. These facts lead us to speculate that the present b-Tub gene may encode the testis-specific isoform.

Publication:

1) Mita, K., Nenoi, M., Morimyo, M., Tsuji, H., Ichimura, S., Sawai, S. and Hamana, K.: Gene, 162, in press, 1995.

4.Mutation Induction by g-rays of Low Dose Rate in Human Lymphoblastoid Cells

Ikuko Furuno-Fukushi, Masahiko Takahagi and Kouichi Tatsumi

Key word: human lymphoblastoid cell, g-rays, low dose rate, mutation induction, hprt

Mutation induction for 6-thioguanine resistance by g-rays at different dose rates was studied in human lymphoblastiod cells, WIL2-NS. Mutation induction showed a curvilinear dose response for acute irradiation (30 Gy/h). The induced mutant frequency decreased after irradiation at 0.17 Gy/h or 0.006 Gy/h compared to that for the acute irradiation. An apparent linear-relation ship between total dose and the mutant frequency was found for the chronic irradiation. No significant difference was found in the mutation frequency as a function of dose between the cultures irradiated at 0.17 Gy/h and those at 0.006 Gy/h. The inverse dose rate effect, which had been observed in proliferating mouse L5178Y leukemia cells, was not observed in WIL2-NS cells so far as at the dose rates employed. Structural alterations at hprt locus in isolated mutant clones were examined with multiplex PCR method and were compared among cultures irradiated at different dose rates. Approximately 15% of spontaneously-arising mutants was accounted for by deletion mutations. When the coexistence of spontaneously-arising mutants in irradiated cultures is taken into account, the highest proportion of deletion mutations (84%) was estimated for g-ray-induced mutants from the cultures irradiated at 0.006 Gy/h (the total dose of 3 to 4 Gy), 60% for those at 30 Gy/h (the total dose of 1-2.5 Gy). These results suggest that low-dose-rate of g-rays preferentially induces deletion mutations at the hprt locus.

5. Effects of gamma-irradiation on the yield of mid-ventral white spots in mice in different genetic backgrounds and at different times during development

Tomohisa Hirobe

Key words: mouse, melanocyte, gamma-irradiation, differentiation, hair follicle, spot

Pregnant females C57BL/10JHir-p/p mice crossed with C57BL/10JHirmales were whole-body irradiated with a single acute dose of 60Co-gamma-rays to investigate the effect of gamma-radiation on embryonic melanoblasts. The effect was studied by scoring changes in the cutaneous coats of F1 offspring 25 days after birth. White spots were found in mid-ventrum of the animals. Melanoblasts and melanocytes were not observed in the spotted skin. The frequency of the spots increased in a dose-dependent manner. White spots were found in mid-ventrum of (C57BL/6J x C3H/HeJmsHir) F1 exposed to gamma-rays. However, the frequency of the spots in (C57BL/6J x C3H/HeJmsHir)F1 wereextremely lower than that in (C57BL/10JHir-p/p xC57BL/10JHir)F1, suggesting the possibility that the frequency of mid-ventral white spots are genetically controlled. Moreover, the highest frequency was found in (C57BL/10JHir-p/p x C57BL/10JHir)F1 irradiated at 8.5 days of gestation. This stage corresponds to the stage of initiation of neural-crest cell migration. These results indicate that gamma-radiation affects the differentiation of melanocytes in the skin both with genetical control and with greater effects seen at the stage of initiation of neural -crest cell migration.

Publications:

1) Hirobe, T.: J. Cell Sci., 107, 1679-1686, 1994.
2) Hirobe, T.: Mutation Res., 322, 213-220, 1994.
3) Hirobe, T.: Histol. Histopathol., 10, 223-237, 1995.

6. Density Dependent Change of Myristoylated Proteins in C3H 10T1/2 Fibroblasts and Their Transformants

Hisaka Sakiyama, Eiko Wada and Shiro Kanegasaki

Various proteins that play an important roles in cellular regulation are kwon to be myristoylated. These include myristoylated alanine-rich C kinase substrate (MARCKS), the alphasubunit of guanine nucleotide-binding regulatory proteins, the catalytic subunit of cAMP-dependent protein kinase, a component of a calmodulin-binding phosphatase, the p56 tyrosine kinase and p60src tyrosine kinase. Although the role of myristic acid in these acylated molecules is not well understood, it is assumed that myristoylation is required both for the association of the protein with memblanes and for full enzymatic activity.

We have examined the pattern of protein myristoylation in C3H10T1/2 fibroblasts during cell growth. During the growth phase of 10T1/2 cells, several proteins were radiolabeled with 3H-myristate, and among them proteins with molecular masses of 22, 35, a doublet of 42-45 and 67kDa were predominantly labeled. The extent of myristoylation in each of these proteins changed with cell density. The amount of the radioactivity incorporated into the 22kDa protein in 10T1/2 cells decreased with increasing cell density and remained at a lowlevel during the stationary phase. In contrast, the incorporation into the 67kDa proteinn increased parallel to cell density. Although 67kDa and 22kDa proteins were labeled with 3H-myristate, the density dependent change of myristoylation was not observed in any of the transformants of 10T1/2 cells thus far examined. The 67kDa protein was identifeid as MARCKS by immunoprecipitation with ant-MARCKS antibody. The amount of MARCKS was revealed to increase significantly in parallel with cell density in 10T1/2 cells but not in transformed cells.

Publications

1) Yamaguchi,K., Kato,N., Sakai,N.,Matsumoto,M.,Nagasawa,S., Hatsuse,H.,Toyoguchi,T., Sakiyama,H.: Biochim.Biophys. Act. 1205,133-138, 1994.
2) Sakiyama,H., Inaba,N., Toyoguchi,T., Okada,Y., Matsumoto, M., Moriya,H. and Ohtsu, H.: Cell Tissue Res.,58, 309-313, 1994.
3) Sakai,N., Kusunoki,M., Nishida,M., Toyoguchi, T., Fukutomi, H. and Sakiyama,H.: Int. J., Cancer, 57, 1-5, 1994.
4) Ichikawa,S.,Nakajo,N., Sakiyama,H. and Hirabayashi, Y.: Proc. Natl. Acad. Sci. USA, 91, 2703-2707, 1994.
5) Toyoguchi, T., Yamaguchi,K., Imajo-Ohmi,S., Kato, N., Kageyama,H., Sakiyama,S., Nagasawa,S., Moriya,H. and Sakiyama,H.: Bichem. Biophys. Act. in press.

1. IL-1 interferes with peripheral tolerance by SEB in vivo

Gen Suzuki, Yukiko Nakata, Akiko Uzawa, and Masayuki Nomura Div. Clin. Res. Radiat. Helth.

Peripheral tolerance is important to prevent autoimmunity of T cells against tissue- specific autoantigens in peripheral organs. In certain pathological situations, peripheral tolerance breaks by unknown reasons and autoimmune diseases occur. Anergy is one of the mechanisms of peripheral tolerance, which down-modulates IL- 2 synthesis and IL-4 responsiveness by helper T cell clones. In this report, we utilized a model system, where Vb8+CD4 T cells were anergized by administration of high dose of Staphylococcus enterotoxin B (SEB) in vivo, and investigated an effect of recombinant human (rh) IL-1 on the tolerance induction. RhIL-1 was used because of its ability to induce IL-4 responsiveness in T cells. In case that rhIL-1 was administered within 24 h after SEB inoculation, the cytokine interfered with tolerance induction; Vb8+CD4 T cells from mice that had been treated with both SEB and IL-1 proliferated in response to SEB and produced IL-2, IL-4 and IFN-g upon TCR/CD28 crosslinking. Delayed administration of rhIL-1 by 48 h failed to do so; T cells did not proliferate in response to SEB, but retained an ability to produce IL-4 upon TCR/CD28 crosslinking. Administration of rhIL-1 induced better proliferation of Vb8+CD4 T cells in response to SEB in vivo, but did not prevent cell death after proliferation. These results suggest a potential role of inflammatory cytokine IL-1 in the course of autoimmunity via interference with tolerance.

References:

1. Suzuki, G., M. Nomura, A. Uzawa, M. Akashi and ,Y. Nakata: Impaired CD28-mediated costimulation in anergicT cells. Int. Immunol. 7: 37-43, 1995.
2. . Nakata, Y., K. Matsuda, A. Uzawa, M. Nomura, M. Akashi, and G. Suzuki: Administration of rhIL-1 prevents tolerance induction by SEB in vivo. J. Immunol. Accepted.

2. Cell-Free Transmission of Fv-4 Resistance Gene Product Controlling Friend Leukemia Virus-Induced Leukemogenesis: A Unique Mechanism for Interference with Viral Infection

Shiro Aizawa, Masanobu Kitagawa*, Hitoko Kamisaku and Toshihiko Sado (*Tokyo Medical and Dental University, Tokyo)

key words: Fv-4r, interference, Friend leukemia virus, bone marrow transplantation

Fv-4 is a mouse gene that dominantly confers resistance to infection by ecotropic murine leukemia virus (MuLV). We previously demonstrated that mixed radiation bone marrow chimeras containing Fv-4r-bearing BALB/c-Fv-4Wr (C4W) bone marrow and Fv-4s-bearing C3H/He (C3H) bone marrow grafted into C3H recipient mice (C4W + C3H ∃ C3H) were resistant to Friend leukemia virus (FLV)-induced leukemogenesis, even when they contained as high as 70% C3H-derived cells. This indicates that FLV-sensitive C3H-derived cells are rendered refractory to infection and/or transformation with FLV when they coexist in mice with Fv-4r-bearing cells. To investigate the mechanism of Fv-4 resistance to FLV-induced leukemogenesis, we first examined the expression of Fv-4r env antigen in the peripheral blood mononuclear cells (PBMC) of these chimeras. The Fv-4r env antigen was present not only on C4W-derived cells, but also on Fv-4s-bearing C3H-derived in C4W + C3H ∃ C3H mixed bone marrow chimeras. The Fv-4r env antigen that binds to the cell surface of C3H cells was found in sera from normal C4W mice. C4W ∃ C3H chimeras, and C4W + C3H ∃ C3H mixed chimeras. The serum Fv-4r env antigen binds to ecotropic MuLV receptors, shown by specific binding to transfectant mink cells expressing ecotropic MuLV receptor, but not to parental mink cells. To determine whether the binding of Fv-4r env antigen to the putative MuLV receptors would block FLV infection, C3H thymocytes or spleen cells that had been preincubated with C4W serum were mixed with FLV and the subsequent expression of MuLV specific antigens was examined. C3H thymocytes or spleen cells treated with C4W serum became refractory to binding by FLV. These results provide evidence that the Fv-4r env antigen is released from C4W-derived cells in vivo and binds to cells expressing surface receptors for ecotropic MuLV, thereby protecting them from infection with FLV.

Publications:

1) Kitagawa, M., Kamisaku, H., Aizawa, S. and Sado, T.: Leukemia, 8, 2200- 2206, 1994.
2) Aizawa, S., Sado, T., Kamisaku, H., Nemoto, K. and Yoshida, K.: Bone Marrow Transplantation, 13, 109-114, 1994.
3) Kitagawa, M., Aizawa, S., Kamisaku, H., Ikeda, H., Hirokawa, K. and Sado, T.: Blood, (in press)
4) Aizawa, S., Kamisaku, H. and Sado, T.: Bone Marrow Transplantation, (in press)

3. The mechanism for inhibition of Radiation Induced Myeloid Leukemia by Caloric Restriction

Kazuko Yoshida, Kumie Nemoto and Tohru Inoue

The spontaneous incidence of myeloid leukemia(ML) is about 1 % in C3H/He mice, and the incidence increased up to about 24 % when whole body irradiation is given with 3 Gy of X-ray. However, the incidence of ML was significantly decreased by caloric restriction(CR), i.e., 7.9 % and 10.7 % in which CR was started at before irradiation(6 weeks old) and after irradiation(10 weeks old) respectively. To explain the mechanism of decreased in the incidence of ML by CR, we, then, analyzed the number of hematopoietic stem cells at the time of radiation which were the target cells of ML.

The calorie-intake was adjusted by controlling the amount of carbohydrate and dextrose. Diets consisted of two different calorie-controlled regimens, i.e., 65 and 95 kcal/week/mouse, but with an equal amount of other nutrients, such as proteins, lipid, vitamins and minerals. The C3H/He male mice in the both control(95 Kcal diet) and restriction group(65 Kcal diet) were fed from at the age of 6 weeks. Then, we examined whether or not the number of target cells would differ between control and restriction groups at the time of radiation(at the age of 10 week).

GM-CFU are granulo and monocytic lineage committed stem cell, and 12d CFU-s are pluripotent stem cell. In the femur, the number of GM-CFU in 105 cells did not reflect any difference between two groups, however, the total number of GM-CFU in restriction groups was significantly lower than control group. On the other hand, in the spleen, the number in 106 cells and total number of GM-CFU in restriction group was decreased less than 10 % of control group. The number of 12d CFU-s in the femur was not different between the two groups. Whereas the number of 12d CFU- s per aliquot spleen cell number in the restriction groups was significantly decreased than the control group. Besides, the total number of CFU-s in spleen was also decreased less than 10% in the restriction groups compared with the control group. From these results, it is clearly demonstrated that the number of hematopoietic stem cells at the time of radiation was significantly lower in restriction group than control diet group. Therefore, the decreased number of target cells may be one of the reasons for the inhibition of radiation-induced myeloid leukemia by caloric restriction. However, the incidence of myeloid leukemia also decreased when the calorie restriction was started after irradiation. In this case, the target cells presented the same number with control group, therefore , we can not explain the number of target cells only. The cell cycle of hematopoietic stem cells may also participate the inhibition of radiation-induced myeloid leukemia. We have been trying to determine this point.

4. IL-1 interferes with peripheral tolerance by SEB in vivo

Gen Suzuki, Yukiko Nakata, Akiko Uzawa, and Masayuki Nomura Div. Clin. Res. Radiat. Helth.

Peripheral tolerance is important to prevent autoimmunity of T cells against tissue- specific autoantigens in peripheral organs. In certain pathological situations, peripheral tolerance breaks by unknown reasons and autoimmune diseases occur. Anergy is one of the mechanisms of peripheral tolerance, which down-modulates IL- 2 synthesis and IL-4 responsiveness by helper T cell clones. In this report, we utilized a model system, where Vb8+CD4 T cells were anergized by administration of high dose of Staphylococcus enterotoxin B (SEB) in vivo, and investigated an effect of recombinant human (rh) IL-1 on the tolerance induction. RhIL-1 was used because of its ability to induce IL-4 responsiveness in T cells. In case that rhIL-1 was administered within 24 h after SEB inoculation, the cytokine interfered with tolerance induction; Vb8+CD4 T cells from mice that had been treated with both SEB and IL-1 proliferated in response to SEB and produced IL-2, IL-4 and IFN-g upon TCR/CD28 crosslinking. Delayed administration of rhIL-1 by 48 h failed to do so; T cells did not proliferate in response to SEB, but retained an ability to produce IL-4 upon TCR/CD28 crosslinking. Administration of rhIL-1 induced better proliferation of Vb8+CD4 T cells in response to SEB in vivo, but did not prevent cell death after proliferation. These results suggest a potential role of inflammatory cytokine IL-1 in the course of autoimmunity via interference with tolerance.

References:

1) Suzuki, G., M. Nomura, A. Uzawa, M. Akashi and ,Y. Nakata: Impaired CD28-mediated costimulation in anergicT cells. Int. Immunol. 7: 37-43, 1995.

2) . Nakata, Y., K. Matsuda, A. Uzawa, M. Nomura, M. Akashi, and G. Suzuki: Administration of rhIL-1 prevents tolerance induction by SEB in vivo. J. Immunol. Accepted.

1. Thymic Lymphomas Induced by N-Propyl-N-nitrosourea in the Rat

Toshiaki Ogiu, Mayumi Nishimura, Hiroko Fukami* and Mutsushi Matsuyama (* Achi Cancer Center Research Institute,** Nagoya University)**

Keywords: chemical carcinogenesis, thymic lymphoma, N-propyl-N- nitrosourea, genetic regulation, BUF/Mna rats, thymic lymphoma susceptible-3 (Tls-3) gene

To clarify the linkage between Hbb and Tls-1 (thymic lymphoma susceptible-1) loci and to investigate other loci concerning thymic lymphomagenesis, the BUF/Mna rat, which is highly sensitive to the lymphomagenic activity of PNU, the WKY/NCrj rat, reported to be resistant, and their cross offspring were used for the present genetic analysis. F1 hybrid and backcross generations were raised from the 2 strains, and 6 genetic markers including Hbb were analyzed in individuals of the backcross generation. However, no linkage between Hbb and Tls-1 loci could be demonstrated since WKY rats also developed a high incidence of thymic lymphomas in response to PNU. Nevertheless, thymic lymphomas developed more rapidly and reached a larger size in the BUF rat case. F1 rats expressed a rather rapid and large tumor growth phenotype, while the [(WKY×BUF)×WKY] backcross generation consisted of rats with either rapid growing or slow growing tumors. It was thus concluded that rapid development of thymic lymphomas is determined by a gene, provisionally designated Tls-3. Analysis of relationship between 6 genetic markers and development of thymic lymphoma in backcross generation demonstrated that the Tls-3 locus is loosely linked to the Gc locus, suggesting a possible location on rat chromosome 14. Tls-3 may not be identical with Tls-1 and other genes concerning thymic tumors, but its relationship with Tls-2 remains obscure.

Publication:

1) Ogiu, T., Fukami, H., Nishimura, M., Matsuyama, M.: Jpn. J. Cancer Res., 86, 638-644, 1995.

2. Age and Radiation Sensitivity of Rat Mammary Clonogenic Cells

Yoshiya Shimada, Jane Yasukawa-Barnes,* Richard Y. Kim,* Michael N. Gould,* and Kelly H. Clifton* (*University of Wisconsin-Madison)

Keywords: mammary clonogen, F344 rats, age, radiation sensitivity

The relative risk of breast cancer is very high among women who were exposed to ionizing radiation during or before puberty. In the current studies, the survivng fractions of clonogenic mammary cells of groups of virgin rats were estimated after single exposures to 137Cs γrays at intervals from 1 to 12 weeks after birth. The radiosensitivity of clonogens from prepubertal rats was high and changed with the onset of puberty at between 4 and 6 weeks of age. By this time, the increase in the size of the clonogenic cell subpopulation was slowing and differentiation of terminal mammary end buds and alveolar structures was occuring. Analysis of the relationship of clonogen survival and radiation dose according to the α/β model showed that the exponential αD term predominated at the second and fourth weeks of age. By the eighth week of age, theβD2 term had come to predominate and survival curve had a pronounced initial convex shoulder. Further experiments are required to determine whether there is an association between the high sensitivity of the prepubertal and pubertal mammary clonogens to radiation killing and a high susceptibility to radiogenic initiation of cancer.

Publication:
Shimada, Y., Yasukawa-Barnes, J., Kim, R.Y., Gould, M.N., and Clifton, K.H.: Radiat. Res., 137, 118-123, 1994.

3. Effects of Low-Dose Prenatal Irradiation on the Central Nervous System (I) Cell Migration in Mouse Cerebral Cortex After Irradiation.

Yasuko Hyodo-Taguchi, Shinji Fushiki*, Chikako Kinoshita*, Yuji Ishikawa and Tomohisa Hirobe

(*Department of Dynamic Pathology, Research Institute for Neurological Diseases and Geriatrics, Kyoto Prefectural University of Medicine, Kyoto 602)

Key words: mouse brain, neuronal migration, prenatal irradiation

Effects of prenatal irradiation on the developing brain have been studied for the past several decades. A particular interest have now been focused on the effects of prenatal irradiation on neuronal migration in the neocortex, because it may be relevant to the pathogenesis of mental retardation seen among A-bomb survivors exposed in utero. In the present experiments,therefore, we investigated effects of ionizing radiation on the migration of neocortical neurons that were produced on embryonic day 14 (E14) mice. The reason we chose the embryonic day 14 as the time point of X-irradiation was that E14 is just in the middle of neuronogenesis in murine cerebral neocortex.

Pregnant mice (C57BL x C3H) were injected with bromodeoxyuridine (BrdU), 0.5 mg dissolved in 0.5 ml saline /mouse, at 9:00 a.m. on 14th day of gestation for labeling S- phase cells. The mice then exposed to 0, 0.1, 0.25, 0.5 and 1 Gy of X-rays. Brains from embryonic day 17(E17) and offsprings at 2(P2wks.), 3(P3wks.) and 8 weeks(P8wks) after birth,were either immersion 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 the BrdU-immunohistochemistry, using monoclonal anti-BrdU antibody (Becton Dickinson) followed by peroxidase reaction visualized with 3, 3'- diaminobenzidine. Immunostained sections were carefully observed under a light microscope and the location of BrdU-labeled cells was plotted onto the 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 the BrdU-labeled cells were seen in the cortical plate of non-irradiated animals as well as of the embryos exposed to 0.1 Gy. On the contrary, in the embryos exposed to 0.25, 0.5 and 1 Gy, the number of BrdU-labeled cells in the cortical plate at E17 was decreased with increasing doses of X- rays, although the number of labeled cells seen in the intermediate as well as in the ventricular zone was increased. In animals at P2wks., P3wks. and P8wks., the distribution of BrdU labeled cells was more confined to the layers IV and II/III in the controls when compared to prenatally X-irradiated animals. Percentage values of BrdU labeled cells in the layer IV of all the prenatally irradiated groups were smaller than those in non- irradiated controls, whereas those in both the layer II/III and V of all the irradiated animals were increased compared to controls. Especially prominent were the effects seen in 1 Gy irradiated animals in which 20 % of BrdU-labeled cells were distributed in the layer V (Fig. 1).

Our observations indicate that (1) the initial migration of BrdU-labeled cells from the ventricular zone towards the neocortical plate was delayed in the embryonic animals exposed to X-rays of 0.25, 0.5 and 1 Gy on E14, and (2) profound effects of prenatal X- irradiation on neuronal allocation in the cerebral cortex were discerned in mature animals.

Legend

Fig.1:

The distribution of BrdU labeled cells in each layer of the the cerebral neocortex.

Animals were X-irradiated on embryonic-14-day and were sacrificed at 2 weeks postnatal.

Figure legend

Fig. 1. Temporal variation of the cumulative relative risks for age-specific mortality from all causes except lymphoma and leukemia after irradiation at day 17 of the prenatal period (-2), or day 0, 7, 35, 105, 240 or 365 of the postnatal period of B6C3F1 female mice with 3.8 Gy gamma-rays from 137Cs.

4. Influence of Age at Irradiation on Temporal Variation of the Cumulative Relative Risk for Age-Specific Mortality

Shunsaku Sasaki

Key words: age-specific mortality, cumulative relative risk, age at irradiation, B6C3F1 mice, gamma rays

Recent epidemiological investigations have suggested that temporal variation of relative risk for development of solid tumors depends on age at irradiation. But it remain still obscure because follow-up period is not enough to elucidate the temporal variation of relative risk.This experiment was carried out in order to examine the temporal variation of of the cumulative relative risk for age-specific mortality from all causes except lymphoma and leukemia. B6C3F1 female mice were irradiated at day 17 of the prenatal period or day 0, 7, 35, 105, 240 or 365 of the postnatal period with 3.8 Gy gamma-rays from 137Cs. All the mice were allowed to live out their entire life spans under a specific pathogen-free condition. Upon death autopsy and histological examination we're carried out. The cumulative relative risk was calculated with standard methods basing on age- specific mortalities.

Cumulative relative risks for age-specific mortality from all causes except lymphoma and leukemia after irradiation at various ages are plotted against the attained age in Fig.

1. Final values of the cumulative relative risks imply life-time risks for radiation-induced increase in age-specific mortality. These results show that mice of the early postnatal and juvenile period are highly susceptible to radiation-induced increase in age-specific mortality and that mice of the middle-age adult period have lower susceptibility. The cumulative relative risks did not remain constant but decreased with age after irradiation at every ages examined. Degree of decrease with age in the cumulative relative risk depended on age at irradiation. Degree of decrease with age was large when mice were irradiated at the fetal, neonatal, juvenile or young adult period; whereas, it was smaller after irradiation at the middle-age adult period.

5. Influence of Reduction of Dose Rate on Tumorigenesis in C3H/He Male Mice.

H.Otsu, T.Furuse, Y.Noda, A.Shiragai and N.Yasuda.

Tumorigenesis was investigated by gamma-ray continuous, whole body irradiation at low dose-rate in C3H male mice, in which Seki et al. confirmed induction of myeloid leukemia by X-ray whole body irradiation on a manner of dose-dependency (Radiat. Res. 1991). Irradiated mice consisted of 13 groups, of which 7 were given doses of 0.25, 0.5,
1, 2, 3, 4 and 5 at a dose rate of 0.882 Gy/min, respectively, and other 6 groups were divided into 2 subgroups by a low dose-rate ; 0.30 mGy/min (L1 group) and 0.016 mGy/min (L2 group) for 22 hours daily and each subgroup was irradiated by a total dose of 1, 2 and 4 Gy. Each group consisted of 250 mice. Unirradiated mice of 500 served as control. Myeloid leukemia and lung tumor occurred statistically significantly among the various types of neoplasms in this experiment. Myeloid leukemia was found at a maximum incidence of 30% in high dose rate groupby 3 Gy, at the incidence of 5% in low dose rate groups by 4 Gy. Lung tumorshowed a maximum incidence of 26% in high dose rate groups by 3 Gy,27% in L1 group by 4 Gy and 25% in L2 group by 4 Gy. The result sugges- ted that effect of reduction of dose-rate on tumorigenesis were different between types of neoplasms.

6. Distribution of Carbon-14 and Associated Radiation Does in Rat Fetal Brain and Liver after Maternal Injection of [14C]Thymidine.

Takahashi, S., Kubota, Y., Koshimoto, C., Sato, H. and Hatashita, S*. (*Jyuntenndo Univ.)

Key words:14C, rat fetus, brain, liver, distribution, radiation dose,

Recently, a highly sensitive imaging plate system has been developed for digital autoradiographic detection of two-dimensional radioactive distribution (for example, Bio Imaging Analyzer, Fuji Photo Film Co. Ltd., Tokyo, Japan). This system allows the sensitive and quantitative detection of radionuclides in histological sections of tiny tissues such as the fetal brain of rodents. In the present study, the regional concentrations of 14C in fetal rat brains after intravenous injection of [14C]thymidine into the mother were determined by using this image-analyzing system. The regional radiation dose from 14C was compared with the average dose for the whole fetal brain as determined by conventional radiochemical measurements.

Pregnant Sprague-Dawley rats were injected intravenously with [14C]thymidine on day 13.5 of gestation, and the concentrations and radiationdoses of 14C in the fetal brain and liver were determined by liquid scintillation counting and autoradiography with imaging plates. The concentrations of 14C in the whole fetal brains determined by liquid scintillation counting were 1.01% of the injected does per gram wet weight at 6 h afterinjection and decreased to 0.39% g− 1 at 48 h after injection. A significant accumulation of 14C was observed in the fetal liver: 3.8 and 0.51% of the injected does per gram wet weight at 6and 48 h after injection, respectively. Autoradiography showed that, especially at earlier periods after injection, there was remarkable concentration of 14C in ventricular zone of the brain and the central region of the liver (Table). With increasing time after injection , the distribution of 14C became relatively uniform. The concentrations of 14C in the ventricular zone of the fetal brain, determined by autoradiography, were much higher thanthose in the whole brain as determined by liquid scintillation counting. Cumulative radiation doses for 6-48 h after injection were 1.27 mGy for the whole fetus and 1.45 mGy for the whole brain. In contrast, the cumulative radiation does for the ventricular zone of the brain which was determined by autoradiography was approximately 2.2 times that for the whole brain.

[Publication]

Takahashi, S., Kubota, Y., Koshimoto, C., Sato, H. and Hatashita, S*.:Radiat. Res., 140, 10-16, 1994. (*Jyuntendo Univ.)

1.Cloning of Stress-inducible Genes of S. pombe.

M. Morimyo, E. Hongo, T. Higashi, K. Mita, K. Sugaya, H. Hama-Inaba and W. Park

Key words; reporter gene, fusion gene library, stress-inducible genes, DNA sequencing

Schizosaccahromyces pombe is one of the most simple eukaryote. In spite of it, it can be aaumed to be a model organism for human, because their genes are similar to human genes which can be normally expressed in yeast and S. pombe is easy to know the function of genes by disrupting them with homologous recombination. To elucidate the repair mechanisms of S. pombe which exhibits extreme resistance to radiation, stress- inducible genes were cloned. Firstly, cloning vector, pYMM5, was constructed by ligation of pBR322 vector with ars gene of S. pombe, URA3, and reporter gene lacZ carrying multi-cloning site on its upstream site. Secondly, the fusion S. pombe DNA library was made by ligation of BamHI and CIAP treated pYMM5 vector with MboI digested and fractionated S. pombe DNA. Cells of a wild-type strain were transformed with these plasmids by Li-acetate or electroporation treatment at a frequency of 106 transformants/mg DNA. Thirdly, transformants exhibiting pale blue color on Xgal plates were obtained at about 1/200, and were replica-plated on Xgal plates. They were treated with various stresses such as UV, X-rays , heat and oxygen radicals. Those colonies which showed darker blue color after stress treatment, were obtained at about 1/100. Plasmid DNA were extracted from 71 candidates and were classified into 26 groups by determining DNA sequences of S. pombe region flanking to multi-cloning site. Many of them were new genes induced by various stresses, but not involved in a typical stress- inducible genes, indicating that they were involved in the later pathways of stress- induction cascade.

2. Haplotype Analysis at the FRAXA Locus in the Japanese Population

Tada-aki Hori, Masatake Yamauchi, Ikuko Kondo*, Robert I. Richards, and Grant R. Sutherland** (*Ehime Univ., **Women's and Children's Hospital, Australia)**

Key words: fragile X syndrome, p(CCG)n repeat, haplotype analysis

The fragile X syndrome is one of the most common genetic disorders and is the most common form of familial mental handicap. Recent studies have shown that the fragile X mutation results from expansion of a heritable unstable DNA sequence, p(CCG)n/p(CGG)n repeat at the FRAXA locus. In general, the p(CCG)n repeat in the fragile X chromosome usually increased in size when transmitted by female carriers, while there is little alteration or reduction in size when transmitted through a male. There is no evidence for any de novo mutation, all probands having parents who themselves have a premutation or full mutation. Linkage analysis of markers in the vicinity of the fragile X mutation has shown that fragile X chromosomes segregate with particular haplotypes in several populations of Europian origin, suggesting that a few founder chromosomes are responsible for the most fragile X mutations in the Caucasian population.
In order to examine the origin of the fragile X mutations in the Japanese population, we have performed haplotype analysis of the FRAXA locus in 40 unrelated fragile X chromosomes and 142 normal chromosomes in Japanese males, by using two polymorphic AC repeat markers, FRAXAC1 and FRAXAC2, which flanks the FRAXA locus. Table 1 summarises the allele frequencies at FRAXAC1 and FRAXAC2 in the Japanese population and compared them with the results in Caucasian populations reported by Richards et al (1992). There were distinct differences in the allele frequencies between Japanese and Caucasians. In normal Japanese X chromosomes, two major haplotypes for FRAXAC1 - FRAXAC2 were DA(50%) and CF(25%), whereas they were CE(48%) and CD(17%) in Caucasians. Two haplotypes, CF(43%) and DB(20%), were more frequent in Japanese fragile X chromosomes than in normal X chromosomes ($\chi 2$, corrected=7.07, $p < 0.008$), while DA haplotype(15%) is clearly under-represented in Japanese fragile X chromosomes. Thus, this analysis provided evidence for founder fragile X chromosomes in the Japanese population, similar to that in Caucasians, although different haplotypes are involved. The distribution of normal allele size of the p(CCG)n repeat among the X chromosomes in the Japanese population is very similar to that reported for Caucasians, except that the most frequent copy number (n=28) is one copy less than that in Caucasians and that there is an additional peak at 35 copies (NIRS-32, 1994). There is significant correlation between FRAXAC alleles and the p(CCG)n repeat copy number in non-fragile X chromosomes. However, in contrast to findings in Caucasian, alleles with more than 31 copies of the p(CCG)n repeat do not segregate with either of the fragile X common FRAXAC haplotypes.

Publication:

Richards R. I., Kondo, I., Holman, K., Yamauchi, M., Seki, N., Kishi, K., Staples, A., Sutherland, G. R. and Hori, T.: Am. J. Med. Genet., 51, 412-416, 1994. ori, T.:

3. Northern Analysis and Gene Mapping of the Mouse Repair Gene, xpg

Yosh-nobu Harada, Yoichi Matsuda, Naoko Shiomi and Tadahiro Shiomi

Keywords:excision repair, XPG/ERCC5, xpg, northern analysis, gene mapping

An intricate network of biochemical repair system has evolved to counteract the deleterious consequences of DNA injury and permanent mutations. One of the major repair processes is the nucleotide excision repair pathway, which is best studied in Escherichia coli. This system removes a broad category of DNA lesions caused by very dissimilar agents such as UV-induced cyclobutane pyrimidine dimers and 6-4 photoproducts, as well as bulky chemical adducts and DNA cross-links. Recently, several genes involved in mammalian excision repair system have been moleculary cloned. A UV- sensitive repair deficient mouse strain, if established, must be very useful for further studying of the nucleotide excision repair mechanism, especially in vivo. For establishing such a strain, we have already cloned several mouse cDNA homologous to the human XPG/ERCC5 cDNA. We propose that the mouse counterpart of the human XPG/ERCC5 gene is named xpg and the locus symbol of the gene is designated as Xpg. Northern blot analysis was carried out to determine the size and tissue transcription specificity of the mouse xpg mRNA. The xpg gene expressed one species of transcript with 4.3 kb at similar levels in the tissues of heart, brain, spleen, lung, skeletal muscle, kidney and testis. The chromosomal assignment of the mouse xpg gene was made by direct R- banding FISH using the xpg cDNA as a probe. The signals were localized on the terminal of R-positive B band of mouse chromosome 1. For refining the localization of Xpg locus, linkage analysis was carried out using the interspecific backcross progeny generated from Mus spretus and C57BL/6. We surveyed the genotype of Xpg locus by southern analysis and of three microsatellite linkage loci, D1Mit18, D1Mit20, and D1Mit22 by Simple sequence repeat polymorphism (SSCP) analysis. The segregation of the xpg phenotype in the 130 backcross mice was shown as three-point crosses with the microsatellite DNA markers as anchors. The Xpg locus was mapped between D1Mit20 and D1Mit18 loci. The genetic dis tance between Xpg and D1Mit20 was 6.2 cM, and between Xpg and D1Mit18 was 2.3 cM.

Publication:

Harada, Y.-N., Matsuda, Y., Shiomi, N. and Shiomi, T.: Genomics, 1995 in press

4.Molecular Cloning of Human DNA Repair Gene ERCC8 I. Construction of Repair Proficient Irradiation Hybrids

Tadahiro Shiomi, Yoshi-Nobu Harada and Naoko Shiomi

Keywords:repair deficient phenotype, ERCC8, irradiation hybrid

To test whether human repair gene(s) can complement the defect in the rodent group 8 UV-sensitive mutant, cell hybrids were constructed between the group 8 mutant and human cells. Since we have never obtained surviving hybrids from fusions of US31 (a group 8 mutant) cells and human cells for unknown reasons, a UV-sensitive partial hybrid named 6L1030 was constructed by fusion of US31 and X-irradiated normal mouse fibroblast line LTA. 6L1030 has a fibroblastic shape and is as sensitive to UV as the parent US31, and was assigned to complementation group 8. 6L1030TGr was fused to human fibroblast WI38VA13. After 14 - 16 days selection with HAT and ouabain, surviving
hybrids were obtained. Half of the hybrid clones tested were resistant to UV irradiation. Hybrid clones were mixed and cultured for 1 month in nonselective medium to eliminate human chromosomes from hybrids. To select UV-resistant hybrids these cells were subjected to five cycles of UV irradiation (5 J/m2) at two day intervals. Surviving colonies were mixed and named 6LH1R. The number of human chromosomes retained in the hybrid cells ranged from 2 to 15. 6LH1R cells were as resistant to UV as control LTA cells. These results indicate that some human gene located on one of the human chromosomes retained in the UV-resistant hybrid cells can compensate the defect in rodent group 8. The gene is designated as ERCC8.

UV-sensitive mutant 6L1030 cells were fused with X-ray-irradiated (60 - 100 Gray) 6LH1R cells. Unfused 6LH1R cells did not survive the X-ray irradiation damage. These cells were subjected to five cycles of UV irradiation (5 J/m2) at two day intervals. 6L1030 cells that did not receive an ERCC8 gene could not survive the UV selection. Five UV- resistant irradiation hybrids were obtained from 5 x 106 6L1030 cells used for cell fusion. These UV-resistant secondary hybrids were named 6LH2R1-5 (clones 1 - 5). X-ray irradiated 6LH2R cells were used as donors in a third round of irradiation hybrid formation and UV selection, and the surviving cells were named 6LH3R. A fourth round produced cell lines named 6LH4R. The pedigree of the irradiation hybrids is shown in Figure 1. The irradiation hybrid cells were as resistant to UV as control LTA cells. Human sequences retained in the irradiation hybrids were reduced by the repeated cycles of irradiation hybrid formation. In 6LH4R clone 1 cells, the length of the retained human sequences was estimated to be about 1 Mbp as judged from the sizes of Alu positive bands. Since no revertants were obtained from different types of control experiments, an ERCC8 gene which complements the defect in 6L1030 should be included in these human sequences.

Figure legend Fig. 1.

The pedigree for the UV-resistant irradiation hybrids. X ray-irradiated cells are marked
with asterisks. Abbreviations: 6TG; 6TG selection, HAT; HAT selection, Oua; Ouabain selection, UV; UV selection, fusion; cell fusion, X ray; X ray irradiation.

1. Life-Span Studies on Carcinogenic Effects of Inhaled or Injected Plutonium in Experimental Rats and Mice

Yoichi Oghiso, Satoshi Fukuda, Yutaka Yamada, Haruzo Iida, Yuji Yamada, Nobuhito Ishigure, Hiroshi Sato, Akira Koizumi and Jiro Inaba

Key words: Pu-239, aerosol, dioxide, citrate, carcinogenesis, dose-effectiveness

For the estimation of lifetime risk of inhaled plutonium to induce lung cancers, female Wistar strain rats were exposed to a single inhalation of a submicron-size but polydispersed aerosol of high-fired 239PuO2. The absorbed lung doses of the exposed animals during their lifetime were estimated in the range of 0.6-12 Gy. As the dose increased, mean survival time was significantly reduced, and cumulative incidence of primary lung tumors was markedly increased as compared to the untreated control animals. While the crude incidence of total lung tumors were the maximum(95%) in the rats received 3-4 Gy, benign adenoma was found at 1.0 Gy or less, increased at 3-4 Gy, but reduced at 5-8 Gy. In contrast, malignant carcinomas including adenocarcinomas, adenosquamous carcinomas and squamous cell carcinomas were not found less than 1.0 Gy, but increased at 5-9 Gy, following slight reduction at 12 Gy. For risk estimation of blood-borne plutonium to induce a variety of tumors, female C3H strain mice were injected with 239Pu citrate, and were examined for incidence of tumors vs. skeletal dose during the lifetime. Although osteogenic sarcomas were not found in the control, their crude incidence increased from 0.06 Gy, reached the maximum(90%) at 6-8 Gy, and decreased in a higher dose range more than 10 Gy. In contrast, the crude incidence of lymphoid tumors was reduced in the dose range of 0.6-5.0 Gy, reached to the bottom at the dose of 6-8 Gy, but increased to 20-30% in a higher dose range of 10-20 Gy as compared to the spontaneous incidence(20%) in the control. Among lymphoid tumors, none of thymic lymphomas but mostly lymphocytic leukemias were observed in the injected animals, while all the lymphoid tumors were either thymic, lymphocytic or histiocytic lymphomas in the control. The other soft tissue tumors were more frequently observed in the control, but were reduced nor observed in the injected animals. None of myeloid leukemias nor myelomas were observed.

Publication:

Oghiso,Y., Yamada,Y., Ishigure,N., Fukuda,S., Iida,H., Yamada,Y., Sato,H., Koizumi, A., and Inaba,J.: J.Radiat.Res., 35, 222-235, 1994.

Oghiso,Y., Yamada,Y., and Iida,H.: J.Radiat.Res., 35, 236-247, 1994.

4. CLINICAL RESERCH

1.Deuterium Magnetic Resonance Imaging of Rabbit Eye in Vivo

Takayuki Obata, Hiroo Ikehira*, Masahisa Koga, Katsuya Yoshida, Tetsuya Suhara, Fukuko Kimura, Yukio Tateno**

(*Chiba University , **Yokohama City University)

Key words:deuterium, magnetic resonance imaging, rabbit eye, water flow

We used deuterium magnetic resonance imaging (2H MRI) to visualize water movement in the rabbit eye. Dynamic 2H MRI was obtained every 3.5 min at 2 tesla by FLASH pulse seuence (TR, 300 ms; TE, 10 ms;α＝90・) with a slice thickness of 10 mm using a surface coil (4 cm in diameter). After topical administration (0.2 ml D2 O), only the aqueous chamber was imaged, and the signals decreased mono-exponentially. The flow rate was 0.113/min, in agreement with that already reported. After intravenous administration of deuterated saline (3 ml/kg), the aqueous chamber became visible first during imaing, then by the vitreous body. The signals around the lens were only faintly detected. Thus, deuterium MRI was determined to be useful for visualizing water movement in the eye.

Publication:

Obata T, Ikehira H, Koga M, et al: Mag Res Med 33:569-572, 1995 Ogino T, Ikehira H, Arimizu N, et al: Annals Nucl Med 8: 219-224, 1994

5. ENVIRONMENTAL SCIENCES

1. Concentrations of Thorium and Uranium in Freshwater Samples Collected in the Former USSR

Kunio Shiraishi, Yasuhito Igarashi1, Masayoshi Yamamoto2, Toshiyuki Nakajima, Ivan P. Los3, A. V. Zelensky3, M. Z. Buzinny3. (1Meteorolog. Res. Inst., 2Kanazawa Univ., 3Ukra.Sci. Cent. of Radiat. Med.)

Keywords: Chernobyl, thorium, uranium, isotope ratios, ICP-MS.

Since the Chernobyl accident much information in many countries has been accumulated concerning radiation protection. Knowing concentrations of radioactive nuclides in drinking water sources in the former USSR is important for dose estimation of its inhabitants. Approximately one hundred freshwater samples were collected in Ukraine, Russia, and Belorussia with regard to the Chernobyl accident. Concentrations of nineteen elements in the same freshwater samples were previously reported (1). In this report, their concentrations of thorium and uranium were determined by both quantitative and semiquantitative analysis modes of ICP-MS. Furthermore, isotope ratios of uranium are discussed from the viewpoints of contamination due to the Chernobyl accident.

The ICP-MS instrument used was a Yokogawa Model PMS2000. Some specifications and operating conditions for the quantitative mode are summarized in our paper (2). A calibration curve was prepared using five concentration levels of the standard solutions, 0, 10, 50, 100, and 1,000 pg/ml, in addition to the internal standard (209Bi). Those of the semiquantitative mode were also reported in a previous report (1).

Concentrations of 232Th and 238U in 102 water samples were determined in the semiquantitative mode without dilution and chemical separation. In a few samples, 232Th was detected. The mean and median were found to be 0.49 + 4.1 ng/ml and non-detectable, respectively. In this statistical calculation, values below the non-detectable level were taken as zero. The global range and median of 232Th in freshwater have been reported as 0.007-0.1 and 0.03 ng/ml. Another study has found a value of 0.1 ng/ml for river water. The level of 232Th in most of the samples analyzed was similar to the global levels except for a few samples (e.g. 40 ng/ml in Krasnodar). For 238U, concentrations ranged from non-detectable to 1,000 ng/ml. Mean and median concentrations were found to be 30.7 + 139 and 0.7 ng/ml, respectively. Literature values of 238U have been reported in the range of 0.002 to 5 ng/ml, and the median was 0.4 ng/ml. Global means, 0.04 and 2.0 ng/ml, also have been reported. The concentration level of 238U in the samples collected in the former USSR was closer to the higher global values. If the freshwater samples in this area had been contaminated, ratios of 234U/238U and 235U/238U would be increased compared with those of uncontaminated areas. Therefore, the isotope ratios of uranium were studied from this viewpoint.

Isotope ratios of 234U/238U and 235U/238U were measured by the quantitative mode. The freshwater samples having 238U concentration above a few ㎍/ml were chosen. A mass spectrum in the profile mode

is shown in Fig.1 for well water collected in Therkassy. Ratios of 235U/238U in the samples analyzed had almost the same values (0.00721 + 0.00006) and were identical to natural abundance (0.00720). Ratios of 234U/238U were found to cover a wide range from 4.55 x 10-5 (radioactivity ratio, 0.83) to 4.38 x 10-4 (radioactivity ratio, 8.0). Radioactivity ratios from 1 to 3 and a global average of 1.9 have been reported in literatures. Although high values were found in several samples, the present mean value, 1.92 was similar to the global values. Those higher ratios of 234U/238U may be explained by the alpha-recoil effect caused on decay of the parent 238U. Relative standard deviations (RSD) of the ratios, 234U/238U and 235U/238U were within + 47% and +17%, respectively. Better RSDs would be obtained by using a longer ICP-MS analysis time. The results show that the high levels of 238U concentration is not due to the Chernobyl accident, but rather to mining activity and geological sources, since the ratios of 235U/238U were not especially higher than that for natural abundance.

In conclusion, no relationship between the Chernobyl accident and high 238U levels in the freshwater samples collected in the vicinity of the reactor was found. The present work is one of the best examples to show that ICP-MS is a versatile analytical method for highly sensitive elemental analyses and comprehensive surveys of elements present in many type of environmental samples.

Publication:

1) Shiraishi, K., Nakajima, T., Takaku, Y., Tsumura, A., Yamasaki, S., Los, I.P., Kamarikov, I.Y., Buzinny, M.G., Zelensky, A.V.: J. Radioanal. Nuc. Chem. Art., 173, 313-321, 1993.
2) Shiraishi, K., Igarashi, Y., Yamamoto, M., Nakajima, , Los, I.P., M.G., Zelensky, Buzinny, M.G..: J. Radioanal. Nuc. Chem. Art., 185, 157-165, 1994.

Fig. 1. Mass spectrum of well water collected in Therkassy, Ukraine.
It was obtained in the profile mode. Integration times for 232Th, 234U, 235U, and 238U were 10, 40, 20, and 10 seconds, respectively.

2. Comparison of Risk Perception on Industrial and Social Events among Three Different Groups of People Who Are Engaged in Education and Research Activities in Japan

Reiko Kanda, Kenzo Fujimoto and Sadayoshi Kobayashi

Keywords: Risk perception, Risk ranking, Japanese public

Risk assessment on technologies and social activities involves subjective judgment as one of its major components, which depends on the perception of risk by individuals. The present study tried to examine degree of difference in risk perception, by means of ranking of risks as perceived by individual person. Subjects were female clerical staffs and researchers who were working in National Institute of Radiological Sciences, and teachers in elementary or junior high schools (see Table). In order to find any effect of time, the results of two separate trials which were made in 1983 and 1992-93 were compared. Subjects were interviewed and asked to rank 30 items of various types of technology and human activities, according to their subjective judgments on the order of perceived magnitude of risk. The order of perceived risks in a group was determined based on the geometric average of rank for each item.
The order of technical risk estimates was obtained as an objective estimation based on the annual contribution to the number of deaths in the U.S.
The rank of perceived risks was found considerably different from that of technical risk estimates. Nuclear power, food coloring, food preservatives, pesticide and antibiotics were judged riskier in general than the objective estimation. It was obviously found in the female group. On the other hand, all groups judged the risks of smoking, alcoholic beverages, electric power, swimming and railroads to be considerably lower than their technical estimates.
There were strong differences in the order of perceived risk among three groups. The female workers viewed food additives and medical supplies, e.g., food preservatives, food coloring, antibiotics and X-rays as riskier than did the researcher group, while vice versa for sports, e.g., mountain climbing and skiing. Perception by the group of school teachers was found to be similar to that of females rather than that of researchers.
The ranks of perceived risk of traffic vehicles were lower in 1992-93 than that in 1983 for both groups of the female workers and researchers. On the other hand, the ranks of nuclear power and X-rays rose significantly during last decade for the female group.
When the accident at a USSR nuclear power plant occurred in 1986, catastrophic on-site situations were repeatedly reported in Japan by various mass media, which should have influenced on public perception of risk of nuclear power. It was also found for the researchers, who were dealing with radiation and were expected to be familiar with radiation health effects, that they judged nuclear power to be riskier than they had judged in 1983.

Publication:

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

3. Accumulation of Radionuclides by Brackish Water Fishes

Motokazu Nakahara, Ryoichi Nakamura and Toshiaki Ishii

Key words: brackish water fish, bioaccumulation parameters, Sr-85, I-125, Cs-137

Uptake and loss of Sr-85, I-125 and Cs-137 by brackish water fishes were observed in the laboratory experiments and the effect of salinity on the accumulation of the nuclides by the fish was also examined. The brackish water fishes used in the experiments were chestnut goby Chaenogobius castanea (average body weight : 1.11±0.19g) and starry flounder Platichthys stellatus (average body weight : 10.3±3.4g). The fishes were collected from a brackish water lake, Obuchi-numa in Rokkasho-mura, Aomori Prefecture where a nuclear fuel reprocessing plant is under construction.

After being acclimatized to the experimental conditions, the fishes were allowed to take up the nuclides in a tank containing the brackish water of Obuchi-numa with the radionuclides for 6 days. Then the fishes were transferred into the non-radioactive brackish water and loss of the nuclides from the fishes was observed for 52 days. Salinity of the brackish water corresponded to about 50% sea water. During the uptake and loss experiments, the radioactivity of each nuclide in the fishes was periodically measured by high purity Ge γ-ray detector equipped with 4000 channels pulse hight analyzer.

Bioaccumulation parameters such as concentration factors at steady state, uptake rate constants, excretion rate constants and biological half-lives of the nuclides by the fishes were estimated by applying exponential functions on the uptake and loss curves of the nuclides. Concentration factors and biological half-lives of the nuclides by the fishes were shown in Table.

To know the effect of salinity on the accumulation of the nuclides by brackish water fish, uptake and loss of the nuclides by chestnut goby were examined in 10, 40 and 80% seawater. In the nuclides, effect of salinity was observed for Sr-85 and I-125 on the accumulation of the nuclides by the goby. The concentration of the nuclides in the fish was enhanced with decreasing salinity.

Table

Concentration factors (CF) and biological half lives (Tb 1 ／ 2) of radionuclides for brackish water fishes (from water at 20℃)

		Sr-85	I-125	Cs-137
Chestnut goby	CF	7.9	50.6	8.6
	Tb1/2(day)	110	24	68
Starry flounder	CF	48.1	13.6	8.5
	Tb1/2(day)	500	11	34

6. APPENDIX

Division of Clinical Research and Radiation Health
Atsuo Akanuma, M.D., Director
Taiko Joshima, Secretary

Radiopharmaceuticals and Radiopharmacology
Osamu Inoue, Ph.D.
Toshiaki Irie, Ph.D.
Kiyoshi Fukushi, M.S.
Masaomi Iyo, M.D.*
Kaoru Kobayashi, M.D.**
Hiroyuki Tadokoro, M.D.**
Takahiro Onoe, Ph.D.**
Syunichi Wakahara**
Hiroki Numba, M.D.**
Takayuki Nakano**
Kenji Mori**

Bio-medical Physics and Engineering
Toru Matsumoto, Ph.D.

Yoshihisa Akiyama, Ph.D.**

Diagnostic Imaging Research
Katsuya Yoshida, M.D., Section Head
Tetsuya Suhara, M.D.

Hiroshi Fukuda, M.D.*
Fumio Shishido, M.D.*
Franck Girard, Ph.D.**
Takayuki Obata, M.D.**
Yasuhiko Sudo, M.D.**
Akira Takami, M.D.**
Yoshiro Ohkubo, M.D.**
Hiroyuki Tadokoro, M.D.**

Kazuhiro Shimada, M.D.**
Shinobu Kitsukawa, M.D.**
Akiyo Aotsuka, M.D.**

Yasunori Imai, M.D.**
Toshiharu Himi, M.D.**
Masato Asahina, M.D.**

Radiation Therapy Research (Experimental Radiotherapy)
Koichi Ando, D.D.S., Ph.D.,
Sachiko Koike
Masashi Kimoto, M.D.**
Shigeru Yamada, M.D.**
Shin-ichi Hori, M.D.**
Keiich Nakagawa, M.D.**

Senior Research Councelor

Clinical Hematology

Yoshiko Kawase
Misao Hachiya, B.S.

Clinical Immunology

Gen Suzuki, M.D., Section Head
Harumi Ohyama Ph.D.
Akiko Uzawa, B.S.
Masako Nose
Harutoshi Kisaki, M.D.**
Yukihisa Miyaji, B.S.**
Yoshiro Aoki, M.D.

Yasuyoshi Watanabe, M.D.
Tomomi Ishidsuki, M.D.**
Keiichi Nakagawa, M.D.**
Shigenobu Kanba, M.D.**
Jun-ichi Ishida, Ph.D.**
Nobuhiko Takai**

Kenjiro Fukuhisa, B.S., Sesction Head

Katsuyoshi Tabushi, Ph.D.**

D.M.Sc., Section Head
Wakako Shimizu, M.D.**
Masayuki Iizuka,M.D.**
Koiji Ono, M.D.**
Takashi Kiuchi, M.D.**
Mayumi Iwakawa, M.D.**
Nobuo Fukuda, M.D.

Makoto Akashi, M.D., Secstion Head

Organization and Staff

Division of Environmental Health　要訂正

Source and Dosimetry of Natural Radiation　第１研究室

Shinji Tokonami, Ph.D.

Internal Radiation Dose Assessment　第２研究室

Yoshikazu Nishimura, Ph.D., Section Head(Since 1995,1 ）　第２研究室長（１９９５年１月より）

Yoshito Watanabe, Ph.D.

Environmental Transfer and Biokinetics 第３研究室

Hiroshi Takeda, Ph.D.

Chemical Behavior of Radionuclides　第４研究室

Senior Research Counselor

Masaharu Okano, Ph.D.

Tetsuo Iwakura, M.S.

Kazuo Watari, Ph.D.