Nagasaki University Global COE Program
Global Strategic Center for Radiation Health Risk Control

ABSTRACTS

International Workshop on

Radiation Epidemiology and Radiobiology

November 29 - 30, 2010

Ryojun Matsumoto Hall
Nagasaki University School of Medicine

ABSTRACTS

International Workshop on

Radiation Epidemiology and Radiobiology

November 29th (Mon)

Opening Address

9:00-9:05 Shunichi Yamashita (Nagasaki GCOE leader)

Session I. Introduction for Mainz-Nagasaki Radiation Research Initiative

Chair: Keiji Suzuki

9:05-9:25 **The Mainz-Nagasaki Radiation Research Initiative**
Hajo Zeeb

9:25-9:45 **Overview of Nagasaki University Global COE Program**
Shunichi Yamashita

Session II. Epidemiological Studies

Chairs: Hajo Zeeb and Vladimir Saenko

9:50-10:20 **Referral of children to computed tomography**
-assessment of medical practice and awareness of health risks among paediatricians, surgeons and general practitioners in Germany
Hiltrud Merzenich

10:20-10:50 **Childhood cancer after exposure to ionizing radiation due to pediatric computed tomographies**
Lucian Krille

10:50-11:20 **Radiation protection in pediatric X-ray CT examinations in Japan**
Keiichi Akahane

11:20-11:50 **Ionising radiation as a risk factor for cataracts**
Gael Hammer

11:50-12:20 **Current status of Cataract Study of A-bomb Survivors**
Kazuo Neriishi

Lunch Break (12:20-13:30)

13:30-14:00 **The role of individual radiation sensitivity in medical diagnosis and therapy-ISIMEP**
Manuela Marron

14:00-14:20 **Molecular signatures of radiation-induced thyroid cancer**
Vladimir Saenko

14:20-14:50 **Patho-epidemiological study in RERF**
Akihiko Suyama

Coffee Break (14:50-15:10)

Session III. Radiation risk and emergency

Chair: Hiltrud Merzenich

15:10-15:30 **Factors influencing perception of radiation risk in people around Chernobyl: a survey in Ukraine**
Yoshisada Shibata

15:30-15:50 **Risk of myelodysplastic syndromes (MDS) in Nagasaki Atomic bomb survivors**
Masako Iwanaga

15:50-16:10 **Evaluation of radiation doses around radiation contaminated areas and development of a novel monitoring devise and system during radiation emergency**
Noboru Takamura

Session IV. Radiobiological Studies (1)

Chair: Motohiro Yamauchi

16:10-16:40 **Identification of genetic predispositions by analyzing the inter-individual variation in the sensitivity of checkpoint and DNA repair after radiation (ISIMEP)**
Steffen Naumann

16:40-17:10 **Studies at radiobiology department of Institute for Environmental Sciences (IES) on biological effects of low dose and low dose-rate gamma-rays on mice**
-How can animal study be useful for reducing the uncertainty of epidemiological study-
Akihiro Shima

Session V. General Discussion (1)

17:10-17:30 Hajo Zeeb and Shunichi Yamashita

Dinner (18:30-21:00; Nagasaki Parkside Hotel)

November 30th (Tue)

Session VI. Radiobiological Studies (2)

Chair: Keiji Suzuki

9:00-9:30 **Mechanical insights into DSB-damage response of human cells after irradiation with different radiation qualities and quantities**
Sandro Conrad

9:30-10:00 **Control of homologous recombination by H2B ubiquitination**
Kenshi Komatsu

10:00-10:20 **Acute DNA damage response and late carcinogenesis in rat thyroid glands after external irradiation**
Masahiro Nakashima

10:20-10:40 **Recognition of chromosome translocation by ATM**
Motohiro Yamauchi

10:40-11:00 **NIPBL is required for cohesin activation in response to DNA double-strand breaks**
Yasuyoshi Oka

11:00-11:20 **Cell cycle analysis during senescence-like growth arrest in human mammary carcinoma cells exposed to ionizing radiation**
Masatoshi Suzuki

Session VII. General Discussion (2)

11:20-12:00 Hajo Zeeb and Shunichi Yamashita

Lunch

The Mainz-Nagasaki Radiation Research Initiative

- Some considerations on international collaboration in radiation research -

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The *Mainz - Nagasaki Radiation Research Initiative* is an international collaboration between the University Medical Center of the Johannes Gutenberg-University Mainz and the Nagasaki University Graduate School of Biomedical Science. The leading institutions are the Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI, Mainz) and the Atomic Bomb Disease Institute (Nagasaki University).

The *Mainz - Nagasaki Radiation Research Initiative* will establish structures to support scientific exchange within research projects, personnel exchange between the partner institutions, and the development of a joint application for an international radiation research group as an instrument to conduct high-level research on radiation and health. The scientific cooperation started its activities in October 2009.The initiative is funded by the German Federal Ministry of Education and Research (BMBF) for at least two years.

Radiation research has long been established as a highly international endeavour, also reflected by well-known international institutions such as the ICRP and others. Also, the nature of radiation exposure with its potential for wide-ranging population effects makes international research collaboration a rather natural development. The presentation will discuss benefits and obstacles for such international collaborations and describe core issues for a successful exchange.

Overview of Nagasaki University Global COE Program

Shunichi Yamashita

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Nagasaki University's Global COE (GCOE) program entitled "Global Strategic Center for Radiation Health Risk Control" has been designed as a world-class academic research consortium intending to establish a pivotal center for education/research with a close focus on radiation-exposed victims such as local populations in Chernobyl, Semipalatinsk and Atomic bomb survivors in Japan and overseas. Facing radiation-related challenges common to the world, GCOE emphasizes education and research in the three disciplines: (1) international radiation health sciences, (2) A-bomb disease medicine and (3) radiation basic life sciences.

The expected results of GCOE's activities are, *in the Scientific Research aspect*: (1) identification of signature gene(s) or sensitive gene groups relating to radiation carcinogenesis; (2) molecular biochemical, molecular pathological and molecular biological characterization of radio-responsive genes involved in chromosomal/genetic instability; (3) elucidation of differences in molecular mechanism of carcinogenesis between early and late carcinogenesis in the same organs in the young exposed; *in the Medical aspect*: (1) planning and implementation of international joint research projects for medical care for Hibakusha, and establishment of the guidelines for radiation safety for low-dose exposure; (2) establishment of comprehensive holistic medical care for radiation victims; (3) development of radiation health risk assessment at the individual levels as well as at the population levels; and *in the Socio-medical aspect:* (1) formulation of the world-standard radiation health risk management program and safety guidelines for radiation exposure, as part of joint programs with WHO, IAEA and collaborating COE research centers in the former USSR countries, and the US, European and Asian counterparts; (2) provision of guidelines in the field of medical radiation exposure and radiation emergency; (3) implementation of education/training program in the field of radiation medicine and establishment a network for radiation emergency response.

As a whole, Nagasaki University's Global COE is an essential and important initiative to promote radiation medical and biological sciences at universities in Japan and overseas countries in a wide range of fields at the global arena. Therefore the international workshop with Johannes Gutenburg University Mainz is very important to promote our joint research projects concretely on Radiation Epidemiology and Radiobiology.

(http://www-sdc.med.nagasaki-u.ac.jp/gcoe/index.html)
(http://www.imbei.uni-mainz.de/nagasaki/)

Referral of children to computed tomography – assessment of medical practice and awareness of health risks among paediatricians, surgeons and general practitioners in Germany

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Objectives: The purpose of a cross-sectional survey among prescribers of CT in children was to assess the frequency of referrals for paediatric CT examinations, the diagnoses and the referral criteria leading to paediatric CT, as well as knowledge and awareness for potential risks of radiation exposure from CT in children.

Background: Computed tomography (CT) plays a major role in image-based diagnostics and is being increasingly utilized. In Germany, CT examinations were responsible for 4% of all radiological procedures in 1994, increasing to 8% in 2007. The estimated annual number of CT examinations rose several-fold between 1994 and 2005: a total of 8,2 Mio CT examinations were applied in 2005 (approx. 100 Ct per 1000 inhabitants) including 0,95% paediatric CTs[1] .The doses of radiation from computed tomography (CT) are relatively high compared to conventional X-ray examinations. However, due to increased radiosensitivity and a longer lifespan, children are at a substantially higher risk for developing cancer as a result of exposure to ionizing radiation compared to adults[2].

Methods: Two study areas have been defined (in the vicinity of two cities in Rhineland-Palatinate and Saxony). A standardized questionnaire has been applied to non-radiologists: all paediatricians and surgeons in the study areas and a random 50%-sample of general practitioners (N=806). Data collection started in November 2009 and has been finalized in February 2010. Study participants have been enrolled in the survey in written form (mailed questionnaire and general information on study aims). Non-responders received one written reminder and will be contacted repeatedly in order to obtain a telephone interview. We achieved a response rate of 36,4% (n=295).

Results: The frequency of computed tomography examinations in CT in children ≤ 15 years was moderate. Referring to the past 24 month 59% of the doctors applied no CT in children and 30% applied only 1-5 CTs annually. A total of 50% of the examined children are older than 10 years. The most frequent indication for a CT examination in children are traumata or suspected cancer diseases. The interviewed participants underestimated the radiation exposure due to CT and they overestimated the radiation exposure due to X-ray examinations. The doctors' estimate of the excess lifetime cancer risk of a 1 year old after CT indicated a limited knowledge regarding potential health risks. Overall, the interviewees were highly interested in guidelines for justification of the CT examination in children. In conclusion, radiological training and education of physicians seems necessary in the light of health risks due to CT in children[3].

Possible joint activities between Mainz and Nagasaki: A joint paper on "The knowledge of radiation dose and awareness of inherent health risks due to CT among physicians in Germany and Japan"

References:

(1) Galansky et al. CT exposure practice in the Federal Republic of Germany : results of a nation-wide survey in 2005-2006. Medizinische Hochschule Hannover 2007

(2) Brenner,D.J., Elliston,C.D., Hall,E.J., & Berdon,W.E. Estimated Risks of Radiation-Induced Fatal Cancer from Pediatric CT. *Am. J. Roentgenol.* **176**, 289-296 (2001).

(3) Heyer CM et al.:Paediatrician awareness of radiation dose and inherent risks in chest imaging studies – a questionnaire study. European J Radiol 2009, doi 10.1016/j.ejrad 2009.06.014

Childhood cancer after exposure to ionizing radiation due to pediatric computed tomographies

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Background: The use of computed tomography is growing in developed countries[2]. Radiation doses from CT are around 10-100 times higher than those from conventional x-ray examinations. CT is currently the major contributor to annual medical radiation exposure and the major reason for the ongoing increase of the exposure[3]. It is well known that cancer risk after irradiation is highest after exposure in childhood as children have a higher susceptibility to ionizing radiation and a longer life span under risk[4]. Brenner et al. estimated that among the 600,000 children annually scanned in the USA, 500 will develop cancer and die in consequence of the radiation exposure from CT[5]. As Rehani and Frush already wrote, there is no controversy on carcinogenic effects for organ doses higher 150mGy[1]. Concerning lower doses only model-based estimations but no primary epidemiological study data are available to estimate the cancer burden from CT exposure. In Germany, we will perform a cohort study of paediatric patients exposed to ionizing radiation through CT with prospective follow-up for cancer incidence.

Study design: Based on the electronically stored patient records from the departments of radiology of large hospitals a cohort of children who underwent a CT examination will be assembled. Records available in the radiology information systems (RIS) include patient identifiers like name, address, sex and date of birth, date and performed procedure. The indication for CT and the report allow identifying those individuals with cancer or other diseases associated to cancer.

Technical information for individual dose reconstruction is stored as metadata together with the image in the picture archiving and communication systems (PACS). This metadata will be abstracted by software specifically designed for this study and the effective dose will be calculated[6]. Individual organ doses will be calculated based on newly modelled conversion factors and having regard of exposed organs.

The full cohort will be pseudonymised and linked with the database of the German Cancer Registry (GCCR). The GCCR was established 1980, registers all cancer cases in children younger 15 years and reached full coverage around 1987. Due to pseudonymisation and strict data separation data privacy is assured.

Statistical analyses will include external comparison (SIR analysis) as well as internal comparison (dose-response analysis). Patients with prevalent cancer will be excluded. Confounding by indication will be a major challenge for the analysis and the interpretation of the study results. Sensitivity analyses will be performed concerning the a priori higher risks of cancer development and mortality for exposed children. Conventional x-rays will not be considered as their dose contribution is considered negligible as compared to CT. Our recent large cohort study did not detect increased cancer risks after conventional x-ray examination7.

Based on calculations from our pilot study we expect to enrol about 80,000 to 130,000 eligible patients in more than 40 hospitals nationwide. Based on the conservative cohort calculation we would be able to detect a SIR of 1.6 for leukaemia and a SIR of 1.3 for all cancer. International pooling to enhance statistical power is envisaged.

The study is funded by the German Federal Ministry of Education and Research. Results are expected in 2013.

Possible joint activities between Mainz and Nagasaki: A first step may be a feasibility study in Japan. The methods and procedures on dose assessment already established in Germany might be applied Japan. During the feasibility study data on study population, examinations and exposure should be collected in at least one hospital. This data may be analysed in respect of examination conduct, average radiation dosages and resulting stochastic risks of late effects. This information can be compared with the German analysis and be presented in an international joined research article. This may increase public awareness and ease further funding in Japan and in Germany.

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3. BfS. Jahresbericht 2007. 2008. Salzgitter, Bundesamt für Strahlenschutz. Ref Type: Report

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Radiation Protection in Pediatric X-ray CT examinations in Japan

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It is well known that the collective doses due to medical exposures in Japan have been very high compared to those in other countries. Actually, for example, there are about 93 CT machines per million of the population in Japan, the highest number among OECD member countries according to the UNSCEAR report (1). However, the important issues on radiation protection are not only the frequencies of radiological examinations but also how to perform justification and optimization considering with both risks and benefits. In addition to that, the protection for pediatric patients is also important because of their radiation sensitivities.

To grasp the current situation on radiation protection, the frequencies of medical radiation uses have been investigated and reported by NIRS, which have been submitted to UNSCEAR as the data of Japan. In terms of the doses of the patients, there have also been many studies by medical professionals in Japan. In one of the study groups of dose estimations, we evaluated the patients' doses on CT examinations by using small dosimeters such as TLDs and photodiode dosimeters and anthropomorphic phantoms of adults and 6-year-old child (2,3). For examinations of 4, 16 and 64 slice CT scanners, organ doses and effective doses were estimated under the practical routine conditions in the medical facilities. For the adult phantom, organ doses and effective doses on chest CT were estimated to be 8-35mGy and 7-18mSv, respectively. On the other hand, for the pediatric phantom, organ and effective doses were estimated to be 4-20mGy and 3-8mSv. The dose variation would be caused by the differences in the types of CT scanners and scan conditions used at each medical facility. The dose measurements with newborn and 1-year-old infant phantoms are now in progress.

In the current situation, the collaborations with persons or organizations involved in radiation protection in medicine have not always been effective, so we established "Japan network for research and information on medical exposures" (J-RIME) on March in 2010, whose secretariat is in NIRS. The J-RIME plays the role as HUB among the organizations and stakeholders of medical radiation, sharing the data on radiation protection and offering the related issues to be discussed. The purposes of J-RIME are to share and provide the evidences, and to act for contributing appropriate justifications and optimizations in medicine.

For improving domestic radiation protection system, we are planning to perform dose and risk estimations on medical radiation as the basis of the protection, especially for pediatric patients collaborating with National Center for Child Health and Development, and other organizations of J-RIME.

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Ionising radiation as a risk factor for cataracts

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Scientific Background

Cataracts, the opacification of the eye lens, are a leading cause of blindness or severe visual impairment, worldwide. Ionising radiation is generally accepted as risk factor leading to the formation of cataracts. Radiation damages the lens cells and studies suggest that dose, dose rate and fractionation have an impact on the latency period and severity of effects. Recent study results indicate that cataract may not be a deterministic effect, but rather a stochastic event following ionizing radiation exposure. We systematically assessed which designs and study populations have been included in published cataract studies.

A systematic literature revealed 67 relevant publications based on 61 studies, most of which describe patient collectives exposed to higher doses of ionising radiation compared to other studies. Only eight studies on workers with protracted exposures were identified, one on workers with acute exposure, six describing populations with either environmental exposure or whose medical exposure was reconstructed by questionnaire, and four publications on atomic bomb survivors. No clear picture of the shape of the dose-response curve for the effect of low-dose ionising radiation on cataract development emerges from the studies published so far. The studies differ in terms of exposure setting, study design, dosimetry, dose ranges, confounders included, and statistical modelling.

Eligible cohorts of workers or population studies with a good dosimetry for ionising radiation, an objective lens opacification measurement and good information on potential confounders are required to detect presumably low risks for cataractogenesis in humans at low dose levels.

On the basis of this information, a study protocol for an epidemiological cataract study is being developed, and respective studies may be conducted using international collaborations.

Current Status of Cataract Study of A-bomb survivors

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We have published three reports[1-3] based on cataract research conducted during 2000 – 2002 and one report[4] based on cataract surgery records. The results indicated that not only posterior subcapsular cataract and cortical cataract but also cataract surgery increase in dose-dependent fashion, with a threshold that could be less than 1 Gy. Such results are the subject of discussion at international organizations responsible for determining international safety standards for radiation exposure. We therefore organized a related workshop titled 'Radiation Cataractogenesis: Epidemiology and Biology' in March 2009.[5]

The current studies indicated below are active.

(1) Analyses on intermediate risk factors and ATM polymorphism
This research is designed to investigate whether there is difference in radiosensitivity among different types of ATM polymorphism.

(2) Cataract prevalence in F1
This study is for investigation into whether there are transgenerational radiation effects on the ocular lens. The study has already been completed and is currently under analysis.

(3) Cataract tissue collection for future studies
Since A-bomb survivors are reaching the peak age for cataract surgery, i.e., around 75 years old, the next ten years will be an extremely important period for such tissue collection. As of October 2010, 26 cataract tissue specimens have been collected and stored. If any molecular analysis projects are proposed based on these tissue samples, new research protocols will be prepared.

(4) Second cataract study during 2010 - 2012
We are conducting ophthalmologic slit lamp examinations among the A-bomb survivors during 2010 - 2012 and comparing the lens images with those obtained during 2000 - 2002 to investigate whether dose-dependent acceleration of cataract development exists in humans.

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The role of individual radiation sensitivity in medical diagnosis and therapy- ISIMEP

Manuela Marron[1] on behalf of the ISIMEP network

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The individual risk of developing cancer increases when humans are exposed to radiation. However we do not know what exactly happens in the cells of the body during this process and what dose of this radiation is safe for the patient. The effects of ionizing radiation in low doses on the human body remain largely unexplored. We suppose that genetic factors have an influence on cellular protection and repair mechanisms, but it is largely unknown to what extents these factors and mechanisms have an effect under lower levels of radiation exposure. The "Intrinsic Radiosensitivity: Identification, Mechanisms, and Epidemiology" research project (ISIMEP) funded by the German Federal Ministry of Education and Research (BMBF) intends to provide some answers.

This interdisciplinary project started in September 2010 and is guided by Mainz University Medical Center in cooperation with the Technical University of Darmstadt, the University of Bremen and the Ludwig Maximilian University of Munich. Approximately 30 scientists, epidemiologists, biologists, physicians, radiation therapists and medical scientists are collaborating within this interdisciplinary network, which link epidemiological, medical, physical and biological research. Within this cooperation, there are the following six molecular biological research projects and two epidemiological studies: 1) Investigation of the molecular response to radiation exposure: the expression of DNA repair genes and DNA damage induced signalling in relation to the intrinsic susceptibility to radiation. 2) Measurement of gamma-H2AX Foci in peripheral lymphocytes after radiotherapy. 3) Metabolic marker of resistance to radiation exposure and genotoxic endpoints. 4) Inter-individual variation in the sensitivity of cell cycle control after radiation. 5) The relevance of the IGF-I receptor for the intrinsic susceptibility to radiation. 6) Identification of genetic predisposition of carcinogenesis related to double strand break repair. 7) Epidemiological cohort study: Childhood cancer after exposure to ionizing radiation due to pediatric computed topographies. 8) Molecular epidemiological case-control study: Cancer in childhood and molecular Epidemiolgie (KIKME).

The molecular epidemiological case-control study KIKME focuses on the contribution of potential genetic risk factors and radiation, identified at the molecular level, to the etiology and prevention of cancer by identifying specific pathways, molecules and genes that influence the risk of developing cancer. Genetic predispositions and biological markers of radiation sensitivity and absorbed radiation doses should be investigated on the population level. The KIKME study is based on the design of a pilot study on DNA expression changes between patients with one neoplasm in childhood and patients with a second primary cancer after a first neoplasm in childhood (GENKIK).

We expect that ISIMEP will be able to answer key questions in the field of medical radiation research. We hope to gain insights that will help to improve radiation protection. Radiation diagnostics and therapy could in theory be tailored to each individual patient with regard to radiation doses. The interdisciplinary approach of ISIMEP is very promising as this research project creates a network of different collaboration partners and enhances their high levels of competence in the field of radiation research.

Financial support: Funding was received from the German Federal Ministry of Education and Research (BMBF) grant no “02NUK016A”.

We would be very interested to conduct a molecular epidemiological case-control study together with Nagasaki. A first step may be a feasibility study in Japan using the established methods from the German study and collect lifestyle information, blood and primary skin fibroblasts from patients with one neoplasm in childhood, from patients with a second primary cancer after a first neoplasm in childhood and from hospital-based controls without any cancer.

Molecular Signatures of Radiation-induced Thyroid Cancer

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Since the phenomenon of radiation-induced thyroid cancer has been widely recognized, especially after the Chernobyl accident, numerous investigations have been conducted to elucidate its distinctive molecular features and to determine the "radiation signature".

The results of mutational studies indicate that the activation of PTC-related oncogenes has proved difficult to assign any one of them a marker specifically associated with radiation-induced cancer. Rather, the prevalence or distribution of oncogenic mutations in radiation-induced and sporadic PTCs associates with patient's age or with the duration of the latent period.

Gene expression profiling proposed the possible existence of molecular classifiers. Although these findings require further validation in the independent series, they suggest that the effect of a physical factor such as ionizing radiation on the thyroid may result in a specific disturbance in gene expressions observed in Chernobyl PTCs.

Recent studies employing comparative genomic hybridization have been successful in identifying oncogene-specific aberrations and indicated that that DNA copy number variations may correlate with radiation exposure and patient's age at exposure.

Molecular epidemiology investigations show that the complex pathways underlying pathogenesis of PTC may be partly shared by the two etiological forms of this thyroid malignancy, such as those involving the *FOXE1* locus on chromosome 9q22. However, genetic components of radiation-induced and sporadic PTCs do not completely overlap, suggesting the presence of other, so far unidentified etiology-specific determinants.

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Patho-epidemiological Study in RERF

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The Radiation Effects Research Foundation (RERF) initiated its pathological research in 1950, and since then has been studying health effects from A-bomb radiation using such pathological samples. Starting in 1990, special focus has been given to histopathological type-based study of certain cancer sites with known association with radiation.

Histopathological type-based studies have been conducted for the breast, liver, saliva, skin, thyroid, ovary, central nervous system, lung, and malignant lymphoma. In addition to a breast cancer subtype study, studies are now being carried out for endometrial cancer and bone/soft tissue tumor.

At RERF, tissue samples embedded in paraffin blocks have long been stored for archival purposes. Due to recent advances in molecular pathology, the importance of paraffin block specimens is ever increasing. Anticipating potential future studies using paraffin block samples in conjunction with the Life Span Study, we are now taking preliminary steps in partnership with Nagasaki University GCOE to develop an archival sample repository system collaboratively with hospitals in the city of Nagasaki. At the symposium, results obtained from our pathological studies to date and future plans will be presented.

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Factors influencing perception of radiation risk in people around Chernobyl: a survey in Ukraine

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Approximately 25 years have elapsed since the Chernobyl accident occurred. The unique health effects having demonstrated a significant association with the radiation exposure by the accident in general residents were thyroid cancers in those exposed to the accident at their childhood. Although most Ukrainian people showed a fear of the Chernobyl accident, it is irony that some people evacuated from the Chernobyl zone have been exposed without notice to more doses of radon in their dwellings.

To elucidate the factors influencing perception of radiation risk in people around Chernobyl, we conducted a survey in Ukraine using self-administered questionnaire consisting of 37 questions including 5 questions about radiation contamination of their living places and foodstuffs, and 9 questions about radiation and risk. The subjects were high school or university students and their parents living in Zhytomyr, Rivne, Kirovograd and Odesa regions, and Slavutych city.

In each of these 5 survey areas, we distributed 330 questionnaires to students and their parents, respectively. A total of 1,536 students (93%) responded to the questionnaire, while in parents only 861 (52%) responded.

In a question about risk perception, we asked the respondents to evaluate each of 11 factors, i.e., flights, smoking, AIDS, traffic accident, water accident, homicide, radiation accident, alcohol, radon in dwellings, fire and narcotics, by 5 categories, (a) no dangerous, (b) just a little, (c) considerably, (d) in a large measure, and (e) greatly dangerous, from the viewpoint of the degrees of danger regarding their own or their relatives' health. For each factor, we compared the frequency of respondents who marked (c), (d) or (e).

In high school or university students, a significant difference by place of residence was observed in smoking, AIDS ($p < 0.05$), radiation accident, radon in dwellings and narcotics ($p < 0.01$), while in parents, was observed in flights, smoking, fire ($p < 0.05$), radiation accident and radon in dwellings ($p < 0.01$). The factors showing a difference between high school students and university students in the association of the frequency with the place of residence were smoking ($p < 0.01$ vs $p = 0.63$), AIDS ($p = 0.03$ vs $p = 0.08$), water accident ($p = 0.04$ vs $p = 0.40$), alcohol ($p = 0.12$ vs $p < 0.01$), radon in dwellings ($p < 0.01$ vs $p = 0.054$), fire ($p = 0.03$ vs $p = 0.37$) and narcotics ($p = 0.080$ vs $p < 0.01$).

The results suggest a significant role of milieu in the risk perception.

Risk of Myelodysplastic Syndromes (MDS) in Nagasaki Atomic Bomb Survivors

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Leukemia is the first malignancy that appeared in atomic-bomb survivors due to the effect of radiation exposure. The significant dose-associated increases in the risk of chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and acute myelogeneous leukemia (AML) were well documented. [1,2] Recently, another type of hematological malignancy, Myelodysplastic syndrome (MDS), is of an increasing concern in atomic-bomb survivors [3]

MDS is a heterogeneous group of disorders characterized by clonal and ineffective hematopoiesis, morphological dysplasia of the blood cells, and an increased risk of developing acute myeloid leukemia. The disease concept was established in 1982,[4] and became considered to be a category of hematological malignancies in 2000[5] because about 25-30% of MDS may progress to AML.

Although MDS and AML are closely related to each other, there are many differences in the clinical characteristics and molecular abnormalities. Therefore, it cannot simply extrapolate the risk pattern of AML to MDS in atomic-bomb survivors. Previous preliminary analyses suggested a possible radiation dose-response increase and inverse distance relationship for MDS among atomic-bomb survivors. [6,7] To investigate comprehensively the MDS risk in atomic-bomb survivors, the Nagasaki University Graduate School of Biomedical Science and the Radiation Effects Research Foundation have jointly conducted a detailed epidemiologic study of MDS since 2004. We confirmed a significant radiation dose-response and inverse distance relationships for MDS and found a different dose-response pattern for MDS from AML. In the symposium, we will demonstrate MDS risk in Nagasaki atomic-bomb survivors based on the latest analysis.

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Evaluation of radiation doses around radiation contaminated areas and development of a novel monitoring devise and system during radiation emergency

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To evaluate current environmental contamination and contributions from internal and external exposure due to the accident at the Chernobyl Nuclear Power Plant (CNPP), we have screened for internal whole-body ^{137}Cs concentrations using a whole body counter (WBC), and measured concentrations of artificial radionuclides in edible mushrooms, soils and stones from each area were analyzed by gamma spectrometry around this area. Furthermore, we have been developing a novel monitoring devices including a bi-axial accelerometer (ACM), thermometer, central processing unit (CPU), memory IC and lithium cell battery, in order to evaluate radiation safety during radiation emergency and to justify the medical radiation exposure to patients. Furthermore, we recently developed a novel monitoring system for the measurement of radiation dose with ACM thermometer, memory IC and lithium cell battery. After its validation we are going to develop this system for the monitoring of nuclear workers, population residing in radio-contaminated areas and patients exposed to medical radiation examination, in order to evaluate their radiation safety and clarify their risk and benefit.

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Identification of genetic predispositions by analyzing the inter-individual variation in the sensitivity of checkpoint and DNA repair after radiation (ISIMEP)

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DNA damage, like DNA double strand breaks (DSBs) induces cell stress that leads to a cellular stress response like DSB repair or cell cycle checkpoints. Defects in these stress responses are often the basis of a genetic predisposition, which can result in a development of malignant tumors. People with such a genetic predisposition generally have a higher risk for developing a tumor than the average of a normal population. A crucial factor in this context is the exogenous induction of DSBs, caused e.g. by ionizing radiation (IR) during radio-diagnostic examinations. The estimation of the inter-individual variation concerning DSB repair and checkpoint control is therefore crucial endpoint for an individual cancer risk estimation and has implications in radiation protection.
During this project, we plan to determine the inter-individual variations of a normal population. In a second step, we want to identify genetic predispositions by measuring the endpoints of DSB repair and cell cycle checkpoint control. Using immunofluorescence microscopy, we will measure the checkpoint capacity and DSB repair sensitivity on the single cell level in different phases of the cell cycle. The use of high doses, that are used in radio-therapeutic applications as well as low doses that are relevant for radio-diagnostic examinations will further give mechanistic insight into the DNA damage response in different dose ranges. The project relies on samples from patients with a primary tumor in infancy and samples from patients which development a second tumor in infancy (GenKIK and KIKME). The cause of the early development of cancer in these patients probably relies on an involvement of a genetic predisposition which we aim to identify and to characterize.
Perspectively, we see a lot of starting points for a Japanese-German cooperation related to methodic aspects and samples recruitment.

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Studies at Radiobiology Department of Institute for Environmental Sciences (IES) on Biological Effects of Low Dose and Low Dose-rate Gamma-rays on Mice

-How Can Animal Study be Useful for Reducing the Uncertainty of Epidemiological Study-

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The Institute for Environmental Sciences (IES) was inaugurated in December 1990 in Rokkasho, Aomori Prefecture to perform empirical studies based on observation and experimentation rather than theory or pure logic on radiation safety of the local public from exposure to low-level radiation. For that purpose, *Low-Dose Radiation Effects Research Facility (LERF)* and *Advanced Molecular Bio-Sciences Research Center (AMBIC)* were established where mice are chronically irradiated with gamma-rays from each 137Cs source 22 hr/day under **SPF** conditions. Three low dose-rates (0.05, 1, 20 mGy/22hours/day) and two medium dose-rates (200, 400mGy/22hours/day) are independently available. The major projects have been 1)lifespan and tumorigenesis in mice continuously exposed to gamma-rays at low dose-rates, 2)dose-rate effect on genomic changes in murine leukemias, 3)adiposity in mice continuously exposed to low dose-rate gamma-ray (non-neoplastic lesion) and 4)dose and dose-rate effects on chromosome aberration frequencies in splenocytes from mice exposed long-term to low dose-rate gamma-rays (1-4).
In my opinion, the relationship between Radiation Epidemiology and Radiobiology has been a long-lasting pending question being primarily asked by epidemiologists. Looking at this issue from a different stand point, we, experimentalists, question ourselves "How Can Animal Study be Useful for Reducing the Uncertainty of Epidemiological Study". I will try to discuss this issue taking our results into consideration.

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Mechanistical insights into DSB-damage response of human cells after irradiation with different radiation qualities and quantities

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Excellent research in the field of the molecular and celluar responses of cells to iononizing radiation is fundamental for the understanding and evaluation of the clinical outcome of tumor patients treated with iononizing radiation (IR), improvement of the radation therapy and risc estimation of radiation exposure to human beings in general. In the last years we investigated DNA-damages response induced by IR over a very broad dose range starting with several mGy up to several Gy and also the effect of different radiation qualities like x-rays, alpha-particles and accelerated carbon ions. In these investigations a variety of different biological endpoints were measured e.g. G1/S and G2/M checkpoint regulation, DNA double strand repair capacity and cell survival. A striking result we obtained by investigating the effect of low-dose irradiation was the complete failure of DSB-repair induced by very low doses IR (1,2). This result has implications for the Linear-No-Threshhold model used for radiation risk estimations.
To get more insights into the repair mechanisms, we determined the different repair pathways in all cell cycle stages and identified factors that are crucial for efficient repair. A surprising result was the identification of a slow repair pathway in G1 and G2 that relies on functional ATM and Artemis (3,4). Although this slow repair component relies on the same composite of proteins, the repair pathway differs between G1 and G2. In G2 we identified this pathway as homologous recombination (4). Strikingly, all DSBs that are repaired by this slow component are located within heterochromatic regions. By depleting KAP1, a heterochromatin building factor, the requirement of ATM and Artemis can be abolished. Further studies of this subset of DSB repair revealed that KAP1 and CtIP, a factor which initiates the resection of a DSB, are phosphorylation targets of ATM (5,6). To gain more insight into the interesting heterochromatic DSB-repair we irradiated mouse cells with carbon ions. A usefull feature of mouse cells is the discrete localised compactation of heterochromatin into so called chromocenters, which can easily be observed. With low angle irradiation, tracks of damaged chromatin can be visualized by γH2AX- or 53BP-staining, thus a spatio temporal analysis of DSB-repair of heterochromatic lesions is possible. A striking result was the fast relocation of DSBs from the initial heterochromatic induction site to euchromatic regions (7). We will present some selected current research topic of our work and looking forward to fruitful discussions.

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Control of homologous recombination by H2B ubiquitination

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Ionizing radiation induces DNA double strand breaks, one of the most serious DNA damage, and hence, NBS1, mutated in Nijmegen breakage syndrome, interacts with ATM and MRE11 to activate cell cycle checkpoint and initiate DNA repair by recruiting their proteins to sites of DNA damage (1-2). Since DNA is packed in chromatin, the chromatin structure should be modified in prior to DNA repair. This dynamic structural change of chromatin is achieved through the modification of DNA-histone interactions, removal of histones from DNA, exchanges of pre-existing histones with new histones, which are regulated by post-translational modifications of histones including ubiquitination, acetylation, methylation, and phosphorylation.

The phosphorylation and ubiquitination of the histone variant H2AX is a well-characterized early response to DNA damage. It occurs minutes after the formation of DNA double strand breaks (DSBs) and leads to enhanced access of repair proteins, including 53BP1, to sites of DSBs. However, it is likely that another histone modification could be involved in this DNA repair pathway, because the localization of RAD51, a key protein in homologous recombination-mediated repair (HRR), to sites of DSBs occurs normally in H2AX-/- cells, which still exhibit significant HRR activity. We demonstrate that, after exposure to ionizing radiation (IR), histone H2B is mono-ubiquitinated independently of H2AX modification and plays a critical role in the RAD51-mediated HRR of DSBs. RNF20, an E3 ubiquitin ligase associated with chromatin reorganization during transcription, forms IR-induced nuclear foci and mono-ubiquitinates H2B to promote recruitment of BRCA1 1 hour post-irradiation. Consistent with these data, depletion of RNF20 and mutation of the ubiquitinated amino acid residue in H2B (K120R) both interfere with the formation of IR-induced foci containing BRCA1/RAD51. Moreover, H2B mono-ubiquitination is followed by the release of H2B and H3 from chromatin. Consequently, RNF20 depletion and the mutation of H2B-K120R result in a pronounced reduction of HRR. The function of RNF20 in promoting HRR is dependent upon its interaction with the C terminus of NBS1. Our data demonstrate the functional importance of H2B mono-ubiquitination as a later response to DSBs and support a model where DSBs lead to the reorganization of chromatin, which in turn regulates the accessibility or retention of factors involved in HRR independently of H2AX.

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Acute DNA Damage Response and Late Carcinogesis in Rat Thyroid Glands after External Irradiation

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Exposure to ionizing radiation (IR) during childhood is a well-known risk factor for thyroid cancer. Among atomic bomb survivors, although a radiation etiology is epidemiologically confirmed in thyroid carcinogenesis, a majority of thyroid cancers are developed in survivors who have been adulthood at the bombing. We have recently demonstrated the induction of DNA damage response (DDR) detected by 53BP1-foci formation and translocated-type genomic abnormalities by FISH in thyrocytes from adult rats after IR. This study analyzed the alteration of histology and expression of DDR molecules in rat thyroid after external IR to further evaluate the radiosensitivity of adult thyrocytes in vivo.

Adult (7 weeks) male rats were exposed to X-ray at doses 8Gy, then, thyroid tissues were removed at 0 to 24 hours, 1 week, 1, 3, 6, 12, and 18 months after IR. The number of Ki-67-positive cycling cells was only less than 1% in non-IR adult thyrocytes. IR-induced apoptosis was not detected in adult thyrocytes by TUNEL methods. In contrast, the number of Ki-67-positive cycling cells was up to 10% in non-IR thyrocytes and gradually decreased to 2% at 24 hours after IR, and numerous TUNEL-positive cells was observed in thyrocytes from young (4 weeks) male rats at 6 hours after IR. We have demonstrated the presence of autophagy in thyrocytes from young rats after IR by electron microscopy. Thus, the radiosensitivity of thyrocytes was higher in young rats than adult rats and mediated through autophagy in acute DDR.

At 18 months after IR, follicular tumor was developed in all of thyroid gland (n=3), suggesting late IR effects on thyroid tumorgenesis in adulthood. FISH analysis revealed a number of both translocated-type and amplification-type signals in tumor cells, suggesting an involvement of DNA double strand breaks (DSB) during thyroid tumorigenesis after IR. Western blot analysis revealed that the level of p53 and p-p53 (Ser15) was increased at 3h after IR, while no significant alteration was observed in the level of p21, puma and cleaved caspase-3 in thyroid tissues from both adult and young rats. These results suggested that IR induced phosphorylation of p53 but didn't activate cell cycle arrest or apopatosis-related downstream effectors in rat thyrocytes. Furthermore, the level of Ku70 expression, which is one of key molecules in non-homologous end-joining (NHEJ) after DNA DSB, was significantly increased in thyroid from adult rats but not from young rats after IR. IR-induced DNA DSB in adult thyrocytes might be repaired by NHEJ, which could be a factor to mediate the radiosensitivity of thyrocytes, subsequently, adult thyroid carcinogenesis as a late effect of IR.

Recognition of chromosome translocation by ATM

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It has been demonstrated that ataxia telangiectasia-mutated (ATM) protein is activated not only by DNA double-strand breaks (DSBs), but also by alterations of higher-order chromatin structure in the absence of DSBs (1). In the present study, we hypothesized that ATM activation might persist long after completion of DSB rejoining, if the DSB-disrupted structure of chromatin flanking rejoined DSB is not properly recovered, and that such case might be more frequent when DSB rejoining occurs illegitimately between different chromosomes. We found that ionizing radiation (IR)-induced foci of Ser139-phosphorylated histone H2AX, whose phosphorylation is mainly performed by ATM, continued to form interstitially on already-rejoined chromosomes, including dicentric/tricentric chromosomes (kinds of chromosome translocation), long (≥15-19 hr) after DSB rejoining. Treatment of ATM inhibitor dramatically elevated the frequency of chromosome translocation appearing in mitosis-progressing cells, which had been irradiated in G0/G1. Induction of p53 siRNA also increased the frequency of chromosome translocation, indicating that ATM suppresses propagation of chromosome translocation through p53-dependent checkpoint. Furthermore, delayed restoration of ATM activity in ATM-inactivated cells harboring chromosome translocation conferred growth disadvantage in such cells. These results indicate that ATM-dependent checkpoint signaling persists in response to misrejoined chromosomes long after completion of DSB rejoining, which minimizes propagation of chromosome translocation.

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NIPBL is required for cohesin activation in response to DNA double-strand breaks

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Cohesin is a chromosome-associated multisubunit protein and mediates cohesion between replicated sister chromatids (1). Cohesin also accumulates to DNA double-strand break (DSB) sites and facilitates DSB repair following ionizing radiation (IR) (2). Association of cohesin with chromatin requires Scc2/Scc4 complex, and this complex is also essential for accumulation of cohesin to DSB sites in yeast (3). However, mechanisms of cohesin loading and activation at DSB sites in human cells are poorly understood. Here, we show that cohesin loading factor NIPBL, the human homolog of yeast Scc2, is required for cohesin-dependent DSB repair. Knockdown of NIPBL abrogated sister chromatid cohesion and NIPBL-depleted cells exhibited premature sister chromatid separation. In response to IR, depletion of NIPBL sensitized human cells to IR and decreased ATM-dependent phosphorylation of SMC1 at Ser957, which is responsible for cohesin-dependent DSB repair. DSB repair in NIPBL-depleted cells, measured by gamma-H2AX/CENP-F co-staining analysis, was impaired in G2 but not in G1 compared to the control cells, suggesting that NIPBL plays a role in homologous recombinational repair (4). Furthermore, NIPBL accumulated to DSB sites generated by UV-C/photo-activating reagents based micro-irradiation (5). The accumulation of NIPBL to DSB sites required MDC1/RNF8-dependent ubiquitin signaling. These data suggest that NIPBL facilitates cohesin activation and contributes to DSB repair after IR.

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Cell Cycle Analysis during Senescence-like Growth Arrest in Human Mammary Carcinoma Cells Exposed to Ionizing Radiation

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Senescence-like growth arrest (SLGA), manifested by irreversible growth arrest, is now recognized as the major cell death mode not only in normal human fibroblasts but also in human solid carcinoma cells after exposure to ionizing radiation. Since normal human cells exposed to ionizing radiation at G1 showed persistent cell cycle arrest, it seems that continuous activation of DNA damage checkpoint is implicated in SLGA. Previously, we also observed SLGA in human mammary carcinoma cells exposed to 10 Gy of X-rays. However, as DNA damage response is defective in cancer cells, how their cell cycle is regulated in the process of SLGA-induction needs to be determined. Hence, we performed live-cell imaging of human mammary carcinoma cells, MCF-7, transfected with Fluorescence Ubiquitin-based Cell Cycle Indicator (FUCCI), which enabled us to visualize cell cycle after X-irradiation.

Live-cell imaging for up to 120 hr after 10 Gy of X-irradiation revealed that more than 90 % of cells exposed at the G1 phase showed persistent growth arrest in G1. About 60 % of them remained as mononuclear cells without cell division, and out of 60 % of cells, approximately 45 % of mononuclear cells persisted in G1 over this time period. However, other 15 % of mononuclear cells continued cell cycling with skipping mitosis. In addition to the mononuclear cells, other 40 % of cells irradiated in G1 did not show cell cycle arrest, and after cell division, the progenitor cells showed multiple micronuclei due to mitotic catastrophe. We confirmed that these cells also persistently arrested in G1. Growth arrest in G1 was also predominantly observed in cells exposed at G2 phase. We found that almost all mononuclear cells skipped mitosis and arrested in the G1 phase. Taken together, our live-cell imaging demonstrated that, while SLGA was induced, many cells continued cell cycle progression, but without cell division in response to ionizing radiation. Thus, the mechanism to sustain cell cycle in G1 plays a quite important function in the process of SLGA-induction in MCF-7.