Radiation Research Institute

Outline and Research Objectives

Radiation Research Institute (formerly called as Radioisotope Research Institute) was established in 1992 for the purpose of radiation safety control of five radioisotope research laboratories belonging to Faculty of Medicine. They are located in basic science buildings, Animal Center, University Hospital, and University Branch Hospital. (The laboratory in University Branch Hospital was closed in March, 2002.) The research is also expected to the Institute on the subjects related to radiations and radioisotopes such as radiation biology, medical application of radiations/radioisotopes, and health physics.

Faculties and Students

Professor and Chair	Kunio Shinohara, Dr. of Medical Science (1996-)
Lecturer	Takahiko Suzuki, Dr. of Pharmaceutical Science

Research Student 1

Past Research and Major Accomplishments

I (K. Shinohara) learned applied physics at undergraduate course. After I got masters degree in biophysics, I took a doctoral course in medical science. In the field of radiation biology, I started my research work on the cytotoxic effects of γ-rays on cultured mammalian cells in a colony state that is a model system for tumor cord [1]. Then the subject moved to DNA damage and its repair induced by radiation and radiomimetic chemicals such as methylazoximethanol acetate, an active principle of cycasin which is naturally occurring carcinogen in cycad, and benzo(a)pyrene. When new radiation source, synchrotron radiation, became available, our research subjects shifted to focus on biological/medical application of monochromatic X-rays such as Auger enhancement of radiation-induced cell death and development and application of soft X-ray microscopy. In the mean time we found that to image hydrated specimens by soft X-ray microscopy at high resolution, we need intense X-ray source to image at a shorter time than the movement by Brownian motion. For this purpose, we applied laser-produced plasma X-rays, by which the exposure time was as short as 300 psec.

1. DNA damage and its repair induced by radiation and radiomimetic chemicals

With the aid of caffeine, we found that methylazoxymethanol acetate induces damage, which is repaired by a mechanism analogous to post-replication repair of UV light-induced damage [3]. In this study, we used synchronized cells obtained by the automatic synchronizer which we developed [2].

In the study of another carcinogen, benzo(a)pyrene, we detected the formation and excision repair of benzo(a)pyrine-DNA adducts in mammalian cells [4, 6]. This adducts were also detected in peripheral human lung tissue when the tissue was cultured in the presence of benzo(a)pyrene [5].

It is also observed that depression level of DNA synthesis is well correlated to the sensitivity of cell death to UV-irradiation in normal human cells and in cells derived from xeroderma pigmentosum patient deficient in DNA repair [8].

2. Radiation- and heat-induced cell death

(1) Heat-induced cell death

Hyperthermia is developed as one of the method for cancer treatment. The sensitivity of cell death induced by the heat-treatment was studied with respect to energy metabolism. It has been demonstrated that when the synthesis of ATP is inhibited, sensitivity of the cell to heat increases [16]. Our results show that ATP level decreases after heat-treatment and in a treatment-time dependent manner [15]. The sensitivity of cells to heat is well correlated to the ATP level of the cells at normal growth condition which corresponds to the pool size of ATP (or potential to synthesize ATP) [26,49]. The results indicate that energy metabolism is a good marker to evaluate heat sensitivity. We also observed that Ga citrate, a tumor-seeking compound, is a good sensitizer to heat treatment [17,47].

(2) Studies on radiation-induced cell death

X-rays inhibit DNA synthesis. Initiation of DNA synthesis is much more sensitive to X-rays than its elongation. We studied carefully this inhibition and resumption of replicon initiation after X-irradiation [7].

Synchrotron radiation is an intense radiation,

intense enough to study the effects of monochromatic X-rays. We first studied an action spectrum of lethal effects on mammalian cells in the wavelength range from 160 to 254 nm. Cells were sensitive to 160 nm and 254 nm and relatively resistant to 190 nm [9]. We found that target site was not DNA but membrane at the wavelength of 160 nm [13].

(3) Auger enhancement

When elements absorb X-rays at the inner shell electrons, they release Auger electrons and are highly toxic. Their LET corresponds to 5 MeV α particles (100 keV/μm). Therefore, efficient induction of Auger effects in cells by X-rays will result in the sensitization to the cells. We studied this enhancement by labeling the cells with 5-bromodeoxyuridine or 5-iododeoxyuridine and monochromatic synchrotron radiations at the wavelengths slightly higher than K absorption edges of these elements (i.e., Br and I). Our results are the first data for the Auger enhancement of cytotoxicity on mammalian cells obtained by synchrotron radiation [10,11]. Auger enhancement is also observed in the induction of chromosome aberration [18]. Theoretical reports pointed the great enhancement and suggested the new possible modality for radiation therapy. However, our experimental results were not in accord with this theory [11]. We re-evaluated this theoretical consideration and found that the theory needs correction. Our correction of the theory fits well to the experimental data [14]. This report [14] is cited in News and Views in Nature by John Humm (336, 710-711, 1988). The results were against the expectation for radiation therapy, but this is science. In the same issue of Nature, another method of Auger enhancement was reported. The method based on the nuclear resonance absorption of the element. Although the results presented in the report could have not been confirmed yet, the idea is attractive. We estimated the enhancement ratio using the same equation as above and found that more than 10 times higher enhancement will be expectable [unpublished results]. However, it is hard to obtain the experimental data to evaluate this theoretical estimate at the present stage. To keep the potential to study Auger enhancement, we tried to detect and characterize Auger enhancement using laboratory X-ray source. We observed small fraction of Auger enhancement that was protectable by cysteamine as a proton donor for radiation damage [41]. We also studied suicide experiment with a radioisotope of iodine-125 [28].

(4) Molecular mechanisms of radiation-induced cell death

Radiation-induced cell death has been classified into two groups: reproductive death and interphase death. Reproductive death is determined by the colony forming ability, while interphase death is thought to be functional loss and estimated by loss of membrane function (as demonstrated by the dye exclusion test) or energy metabolism. We first studied the difference in these two modes of cell death. For this purpose human leukemic MOLT-4 cells are well suited. They show interphase death at relatively low dose irradiation and also are able to be determined for the colony forming ability. The data [29] show that both types of cell death occur in MOLT-4 cells and that the mechanism of cell death is apoptosis. Next, we compared MOLT-4 with M10, which is a radiosensitive mutant of L5178Y derived from mouse lymphocytic leukemia and has the same level of radiosensitivity. Unexpectedly, we found that M10 developed necrosis, while MOLT-4 died by apoptosis at the same dose level of irradiation [31]. It is confirmed with the heat-treatment that M10 has a system to induce apoptosis (, which is not working at X-irradiation,) probably by the different mechanism from MOLT-4 [31,42].

Based on these results, we are interested in the molecular mechanisms of radiation-induced apoptosis (MOLT-4) and necrosis (M10). Now we are focused on the apoptosis in MOLT-4 cells. (Because of the lack of manpower, radiation-induced necrosis in M10 is remained to be studied in future.) Radiation-induced apoptosis in MOLT-4 cells is p53 dependent [45,48] and caspase-3 dependent [50]. It should be noted that inhibition of proteasome also induced apoptosis in MOLT-4 cells probably with the different mechanism from X-rays because both apoptosis were additive [39].

3. Development and application of soft X-ray microscopy

Soft X-rays in a wavelength range of 1-10 nm are provided by synchrotron radiation and moderately absorbed by biological materials. Soft X-ray microscopy has following advantages over optical and electron microscopy: higher penetration depth than electron microscopy and higher resolution than optical microscopy. Therefore, with soft X-ray microscopy, it is expected that we can observe a whole live cell at higher resolution than optical microscopy. When we started this project, there was no soft X-ray microscope. And now, internationally, there are some microscopes with the potential to image at a resolution of 20 nm. However, unfortunately, no active microscope at high resolution has been working in Japan. This is the present stage. More than 15 years ago, we were interested in soft X-ray microscopy and started to study the importance of this new method in biology.

When we started, the only method available for experimental test was a contact microscopy using a

thin film of polymethylmetacrylate (PMMA) that is developed and characterized by IBM for industrial purpose. We studied and applied it for imaging human cells and chromosomes with some improvement of the method for the observation of PMMA by a transmission electron microscope [12]. We have succeeded in the observation of chromosome fibers and nucleosomes [19-21,33]. The results (Fig. 1) were top data with respect to the resolution to image biologically meaningful specimen and were cited in books from USA, China and Japan. Then we studied the condition to image hydrated biological specimens including the problem of radiation damage and image blurring by Brownian motion [22,24,25]. The conclusion of this study proposed the use of short pulsed X-rays for imaging intact specimens in hydrated condition with successful results for hydrated chromosomes with a single shot exposure of laser-produced plasma X-rays at the exposure time of 300 picoseconds [23,30,32]. However, our results were obtained by contact microscopy and the method is good for molecules but not well suited for imaging inside a whole cell. Therefore, we proposed a possible microscope system to image whole cells [27]. The system is now in the stage of development by other groups.

Another important factor for the application of soft X-ray microscopy to biological/medical science is the contrast of the images for thick specimens. To overcome this problem, specific staining with three-dimensional observation may be the best choice. In the case of soft X-rays, there is another possibility to identify elements and molecules: spectroscopic analysis of imaging (spectromicroscopy). We have been working on spectromicroscopy in two aspects: instrumentation [43,44,46] and application [34,37]. This line of work is in progress. For three-dimensional imaging, we have also studied on holographic microscopy [35,36,38,40] and found that even with holographic observation, three dimensional reconstruction may not be possible with a single exposure.

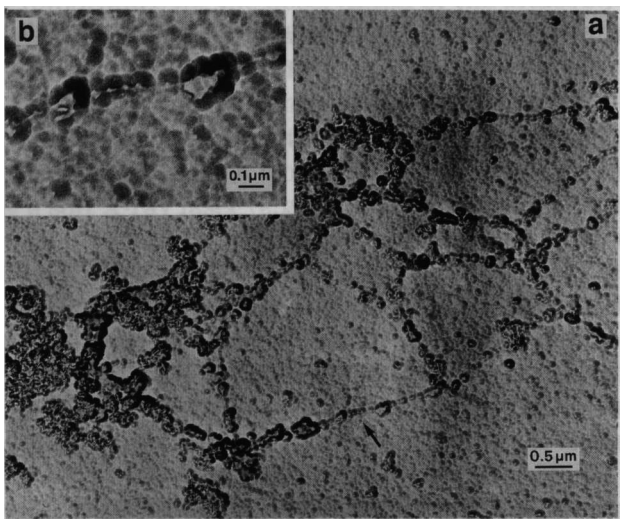

Fig. 1. X-ray image of a stretched portion of a human chromosome [21]. An enlargement of the area indicated by an arrow in (a) is presented in (b).

Current Research

1. Radiation-induced cell death

(1) Molecular mechanisms of radiation-induced apoptosis

Our present study is focussed on the switching mechanism of p53 to select DNA repair or to decide suicide. For this study we are currently collecting the data for p53, and related molecules and phenomena in each single cell to get the idea what is going on in an individual single cell.

(2) Auger enhancement

As shown above, theoretical consideration shows that Auger enhancement following nuclear resonance absorption is an attractive method. Although we have an idea to evaluate the theoretical results experimentally, we have no clue to apply this method for clinical tumor therapy. Therefore, the work remains in theoretical level at the present stage.

(3) Cytotoxic effects of ultrahigh dose rate X-rays

Time scale of radiation effects on life begins with physical interaction (10^{-18}-10^{-15} sec) followed by chemical and biological processes. At a conventional method of X-irradiation, these physical and chemical processes will happen dispersively and induce ionization as one after another. In contrast, particle beam densely ionizes irradiated materials and gives increased cytotoxicity to cells. It is of interest to see what will happen if one can concentrate ionization by X-rays in a short instance especially in shorter than one picosecond. Since such a radiation source is available in recent years, we started to study the cytotoxic effects of ultrahigh dose rate X-rays on mammalian cells at the Institute of Laser Engineering, Osaka University. Because of limited beam time, we are gradually correcting results and have no publication so far, though five years have already passed.

2. Development and application of soft X-ray microscopy

Currently our subject is focussed on spectromicroscopy of the cell to understand images for the improvement of contrast. We have collected images of a cell at various wavelengths in a wide range (1-10 nm). With absorption characteristics of elements and molecules, we are trying to identify these elements and molecules using computer-assisted image process-

ing. The work is in collaboration with Prof. Ito of School of Engineering, Tokai University.

Future Prospects

1. Radiation-induced cell death

(1) Auger enhancement

It is not clear that Auger enhancement can be applied for radiation therapy. However, I believe it is worth to continue studying.

(2) Ultrahigh dose rate X-rays in biology and medicine

The new radiation source may be a useful method for discovering the new mode of diagnostics and radiation therapy. At present the work is in the beginning stage. We will keep working on it.

(3) Molecular mechanisms of radiation-induced apoptosis and necrosis

We are planning to understand the full scheme of the flow of signal transduction process in an individual cell from DNA damage induced by X-rays to the development of apoptosis. The results will contribute to understand the whole scheme of apoptosis and to explore the new mode of radiation therapy with the induction of apoptosis.

2. Soft X-ray microscopy

We believe that soft X-ray microscopy will be established and applied to biological/medical research as a complementary method to optical and electron microscopy in future. The system will become compact and stay in laboratory in the size comparable to a transmission electron microscope.

Research Grants

1. Grant-in-Aid for Scientific Research (B) (1) [2001-2003] Studies on biological effects of ultrahigh dose rate pulsed X-rays.
2. Grant-in-Aid for Scientific Research (A) (1) [1997-2000] Development and its medical/biological application of a projection X-ray microscope using synchrotron radiation.
3. Grant-in-Aid for Exploratory Research [1997-1998] Studies on cytotoxic effects of ultrahigh dose rate X-rays.
4. Grant-in-Aid for Scientific Research (A) (1) [1995-1997] High resolution imaging analysis of elements and molecules in a cell with an X-ray contact microscope.

Select Publications

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