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College of Physicians and Surgeons
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THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY – an NIH-Supported Resource Center

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Collaborating Departments and Institutions

Individuals from the following departments and institutions (listed alphabetically) collaborated with Center for Radiological Research staff in the above research abstracts (for individual attributions see specific reports):

Collaborating Columbia University Departments:
- Department of Ophthalmology
- Department of Urology
- Herbert Irving Comprehensive Cancer Center and Department of Biomedical Informatics, Columbia University Medical Center.

Collaborating Institutions:
- Brookhaven National Laboratory, Biology Department, Upton, NY
- International Space Radiation Laboratory, National Institute of Radiological Science, Chiba, Japan
- Radiation Effects Research Foundation, Hiroshima, Japan
- Southern Urals Biophysics Institute, Ozyorsk, Russia
- Stuyvesant High School, New York, NY (student participating in our Small Group Apprenticeship program)
- University of California, Berkeley, CA
- University of Tarapaca and Research Center for the Man in the Desert, Arica, Chile
- US Department of Health and Human Services
  - National Institutes of Health
    - National Cancer Institute, Division of Basic Science, Bethesda, MD

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Federal:
- Department of Energy
  - Office of Energy Research
  - Office of Environment, Safety and Health
  - Office of Health Programs
- Department of Health and Human Services
  - Health Resources and Services Administration
  - National Institutes of Health:
    - National Cancer Institute [Program Project (PO1) & Individual Research Grants (RO1s)]
    - National Institutes of Environmental Health and Safety (RO1s)
    - National Institute of General Medical Sciences (RO1)
    - National Institute of Bioimaging and Bioengineering (P41)
    - National Center for Research Resources (S10)
  - National Aeronautics and Space Administration

Private:
- Lance Armstrong Foundation
- Radiological Society of North America
- Ruth Estrin Goldberg Memorial for Cancer Research

Web Sites

- Center for Radiological Research ................................................................. http://crr-cu.org
- Radiological Research Accelerator Facility .................................................... http://www.raraf.org
- Web-Rad-Train .......................................................................................... http://www.web-rad-train.org
- Department of Radiation Oncology ............................................................. http://cpmcnet.columbia.edu/dept/radoncology
- Radiation Safety Office .............................................................................. http://cpmcnet.columbia.edu/dept/radsafety
- CRR Annual Reports (1998-present; any corrections will be posted here) ........ http://crr-cu.org/reports.htm
At approximately one month intervals during the academic year the Center for Radiological Research is pleased to welcome accomplished specialists from around the world to present formal seminars and/or spend time discussing ongoing research. The seminars are attended by Center and RARAF professional staff, senior technical staff and graduate students, as well as doctors and scientists from other departments of the College of Physicians & Surgeons interested in collaborative research. Attention has focused on recent findings and future plans, with special emphasis on the interdisciplinary nature of our research effort.

The 2004 sessions, which were organized and scheduled by Dr. Yongliang Zhao, included the following guest speakers (listed alphabetically):

- Dr. Yuri Dubrova, Dept. of Genetics, University of Leicester, UK, “Radiation-Induced Transgenerational Instability in Mice: A Story of Three Generations.”
- Dr. Robert Ullrich, Dir. of Research, Animal Cancer Center, Colorado State University, Fort Collins, Colorado, “Genomic Instability and Radiation Induced Cancer.”
- Dr. Zigang Dong, Hormel-Knowlton Professor, The Hormel Institute, University of Minnesota, Austin, MN, “The role of MAP kinases in Cancer Development and as Target for Cancer Prevention and Is Frequently Downregulated in Primary Human Lung Cancer.”
- Dr. Victor Fung, Scientific Review Administrator, Cancer Etiology Study Section, Center for Scientific Review, National Institute of Health, Bethesda, MD, “Grantmanship in a Highly Competitive Era.”

Seminars were also conducted by our own Center for Radiological Research staff members:

- Dr. Sally Amundson, “Functional Genomics for Radiation Response and Bystander Studies.”
- Dr. Adyabala Balajee, “Induction of Base Excision Repair Proteins in Bystander Cells.”
- Dr. David J. Brenner, “Cancer Risks at Low Radiation Doses—What Do We Really Know?”
- Dr. Guy Garty, “Building a Microbeam Irradiator without an Accelerator.”
- Dr. Haiying Hang, “9-1-1 Checkpoint Complex and S/M Checkpoint Control.”
- Dr. Tom K. Hei, “Oxidative Stress and Environmental Carcinogenesis.”
- Dr. Vladimir Ivanov, “Arsenite-Induced Signaling at the Crossroad of Cell life and Death.”
- Dr. Gerhard Randers-Pehrson, “Status of the New Microbeam at RARAF.”
- Dr. Yongliang Zhao, “Betaig-h3 Gene Mediates the Tumor Suppressor Function of TGF-Beta1.”

(L-r): Dr. Peter B. Schiff (Department of Radiation Oncology), Dr. Charles R. Geard, Dr. Steven R. Isaacson (Department of Radiation Oncology) and Dr. Tom K. Hei.

Dr. Sally A. Amundson and Mrs. Margaret Geard.
The Center for Radiological Research of Columbia University pursues a multidisciplinary approach to understanding the biological effects of a spectrum of ionizing radiations. There are a number of diverse research goals:

- Understanding the mechanisms of the biological effects of radiation at the molecular level.
- Investigating the deleterious effects of low doses of radiation, of interest to the Department of Energy Low Dose Program.
- Investigating the biological effects of high energy heavy ions, of interest to NASA.
- Improving dose-time schedules for clinical radiation therapy and estimating the impact of radiation induced second malignancies.

This report summarizes the principal research initiatives and academic activities during the past year.

The Center received a great deal of publicity as a consequence of a paper published by Dr. Brenner and Carl Ellis-ton estimating the risks associated with whole body CT scans. The findings were reported in newspapers from many parts of the World, and Dr. Brenner was invited to appear in numerous radio and television programs.

The Columbia microbeam continues to be a major research resource for scientists in the Center, as well as for colleagues from other institutions.

Dr. Howard B. Lieberman’s group moved forward with research related to Rad9, and published several related papers, including one that described the construction and analysis of Mrad9 knockout mouse cells and whole animals. In addition, together with Dr. Yin’s laboratory, they demonstrated that human Rad9 could regulate the transcription of p21. Dr. Lieberman also edited a book, entitled “Cell Cycle Checkpoint Control Protocols.”

Through a training program established with the Chinese Academy of Sciences in 1995, our second Columbia University-trained Chinese doctorate student, Ann Xu, successfully defined her Ph.D. thesis in May, 2004. She was awarded her degree in July. Dr. Xu obtained a Master of Public Health degree from Columbia University and conducted her thesis work in Professor Tom K. Hei’s laboratory.

Using the Columbia University microbeam, Dr. Tom K. Hei and his team have shown that targeted cytoplasmic irradiation can induce bystander genotoxic response in both hamster cells as well as in primary human bronchial epithelial cells. This work is supported by a new NIH grant.

A new line of immortal human small airway epithelial cells has been established in Dr. Hei’s laboratory using ectopically expressed telomerase. These cells, which took more than two years to establish, are chromosomally stable and will provide a valuable model for studying radiation and environmental lung cancers.

Recently the microbeam was used by researchers from Dr. William Bonner's lab at NIH in collaboration with Dr. Lubomir Smilenov from CRR. The scope of the project was to estimate the probability of bystander effect based, DNA double strand break induction. The combined expertise of the researchers from both institutions in microbeam techniques and detection of DNA dsb foci produced interesting results. For first time it was shown that bystander effect factors could induce DNA double strand breaks in non-irradiated cells.

The productivity of the Center continues at a high level, as evidenced by a steady stream of scientific papers in peer-reviewed journals, including several in high profile journals. Members of the staff are frequently invited to participate in national and international meetings, and are frequently called upon to serve as consultants, reviewers or site visitors by government and private agencies.

The teaching activities of the Center include the teaching of radiation biology and radiation physics to undergraduates, medical students, and graduate students in the School of Public Health, and to residents in both Radiology and Radiation Oncology, as well as a City-wide course for residents in Radiology.

The Center’s particularly successful Web-based course, “Web-Rad-Train” (http://www.web-rad-train.org), sponsored by the Radiological Society of North America, caters to residents in Diagnostic Radiology and Nuclear Medicine preparing for their board examinations. Thus far nearly 1,300 students have registered to use the web site, which accumulated over 277,000 hits in 2004 and 640,000+ hits since its inception.
Faculty and Staff

FACULTY

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Faculty and Staff

Front row (l-r): Dr. Howard Lieberman, Dr. Tom Hei, Ms. Monique Rey, Dr. Eric Hall, Ms. Mary Coady, Dr. Charles Geard, Dr. David Brenner, Dr. Sally Amundson.

2nd row: Dr. Jaime Rubin, Dr. Wenhong Shen, Mrs. Cui-Xia Kuan, Ms. Sarah Huang, Ms. Xiaojian Wang, Dr. Corrine Leloup, Dr. Aiping Zhu, Mr. David Cuniberti, Ms. Gloria Jenkins-Baker, Dr. Catherine Mitchell, Dr. Su-Xian Liu, Ms. Heidy Hernandez, Ms. Diana Morrison, Dr. Hongning Zhou, Mr. Alan Wong, Dr. Koon Siew Lai, Mr. Gary Johnson.

3rd row: Dr. Rudranath Persaud, Mr. Ronald Baker, Dr. Adayabalam Balajee, Dr. Genze Shao, Dr. Jianli Wang, Mr. Jaeyong Ahn, Dr. Alan Bigelow, Dr. Guy Garty, Dr. Chang-Qing Piao, Dr. Lubomir Smilenov, Mr. Joseph Gillespie, Dr. Vladimir Ivanov.

Back row: Dr. Yuxin Yin, Dr. Yong-Liang Zhao, Mr. Moshe Friedman, Dr. Rajamanickam Baskar, Mr. Robert Archigian, Mr. Kevin Hopkins, Mr. Gregory Ross, Mr. Carl Elliston, Dr. Gerhard Randers-Pehrson, Dr. Giuseppe Schettino, Dr. Stephen Mitchell, Mr. Stephen Marino, Dr. Brian Ponnaiya.

Not pictured: Dr. Gloria Calaf and Ms. Angela Lugo.
Staff News

Dr. Eric J. Hall and Dr. David J. Brenner are both Councilors of the National Council on Radiological Protection. Dr. Brenner serves on NCRP Committee 12, on the use of ionizing radiations to combat terrorism.

Dr. Hall and Dr. Tom K. Hei presented papers at the International Space Research (COSPAR) meeting held in Paris in July.

Dr. Howard B. Lieberman is currently a member of the basic and preclinical Subcommittee C of the National Cancer Institute Initial Review Group. In addition, he is a member of the Scientific Advisory Board for the Israel Cancer Research Foundation. Dr. Lieberman also served as an advisor for the Pennsylvania Department of Health performance reviews, and as an ad hoc reviewer for several other agencies.

Dr. Tom K. Hei continues to serve as an ad hoc member of the NCI cancer etiology study section and as chairman of several special emphasis panels.

Dr. Hei delivered the keynote lecture entitled “Oxidative stress and environmental carcinogenesis” in June at the Asbestos and Nanoparticle meeting held on the campus of the 600 years old University of Torino in Italy. Dr. Hei also gave the Rudbeck Lecture at the annual Nordic Radiobiology meeting in Uppsala, Sweden.

Personnel changes at the Center included the following:

- Dr. Haiying Hang, Assistant Professor of Radiation Oncology, has left the center to assume a new position at the Chinese Academy of Sciences, Beijing, China.
- Dr. Fu-ru Zhan, Post-Doctoral Research Scientist, has also left the Center to assume a new position at the Hefei Institute of Physical Sciences, Hefei, China.
- Dr. Gloria Calaf is now an Adjunct Associate Research Scientist at the Center, in addition to being a professor at the University of Tarapaca in Chile, where she spends half the year.
- Drs. Catherine R. Mitchell and Stephen Mitchell were promoted from Post Doctoral Research Scientists to associate research scientists.
- Dr. Wenhong Shen is a new Post-Doctoral Research Scientist working in Dr. Yin’s lab.
- Dr. Genze Shao is a new Post-Doctoral Research Scientist working in Dr. Hei’s lab.
- Dr. Giuseppe Schettino is a new Post-Doctoral Research Scientist working at RARAF.
- Jaeyong Ahn is a new Staff Associate working in Dr. Amundson’s lab.
Proposed Multiphoton Microscope for the Columbia University Microbeam II Endstation

Alan W. Bigelow, Gregory J. Ross, Gerhard Randers-Pehrson and David J. Brenner

Studies at Columbia University’s Radiological Research Accelerator Facility (RARAF) concentrate on fundamental investigations into the radiobiological effects on mammalian cells through controlled single-cell single-particle microbeam irradiation. Some of the facility’s prime directions are to develop new techniques for imaging cells, to create new tools for the detection of single-molecule events in living cells, and to generate new strategies for dramatically increasing the resolution of imaging dynamic cellular processes. The first-generation irradiation platform at RARAF, microbeam I, was an apertured ion beam; its infrastructure concepts were incorporated by Microbeam II, the facility’s present cell irradiator, that now delivers an ion beam produced by a 4.2 MV Van de Graaff accelerator and focused by an electrostatic lens system. The heart of the microbeam II endstation is a Nikon Eclipse E600-FN research fluorescence microscope.

In order to detect and observe the short-term molecular kinetics of responses to radiation in single living cells within 2D and 3D scenarios, we plan to combine a multiphoton microscope with a single-cell single-particle microbeam irradiator. A multiphoton microscope is a 3D, non-destructive imaging tool that, when compared to conventional confocal microscopy, has greater penetration depth and has reduced phototoxicity and photobleaching in the sample bulk. The guiding principle of the multiphoton microscope is: when two photons are spatially and temporally coincident within the excitation cross section of a fluorophore molecule, they can act as one photon with twice the energy to excite an electronic transition. An aspect of our custom design is that the microscope is mounted on a pivot arm where it can be operated in an on-line or off-line mode. In the off-line mode, the multiphoton microscope is placed above an additional photo-multiplier tube (PMT) which increases light-gathering efficiency and offers particular benefits for transmitted signal from second harmonic generation (SHG), a second-order nonlinear optical process, occurring at regions or interfaces that lack inversion symmetry and is complementary to multiphoton excitation. A schematic view of the major components of our multiphoton microscope design is shown in Figure 1.

On the microbeam II table, pulses from a tunable titanium sapphire laser exit the light guide, are deflected by x-y scanner mirrors in the scanning head into the microscope, and reflect off a low-pass dichroic mirror down to the back aperture of the objective lens, as shown in Figure 2. Multiphoton excitation and SHG occur in a thin layer of the sample at the focal plane of the microscope objective. Fluorescent light emitted isotropically can be collected by as-

Fig. 1. Schematic layout of the RARAF endstation. A silhouette of the multiphoton microscope and SHG imaging system is shown in the online position above the beam port through the microbeam II table. The scanning light source is a tunable, scanning mode-locked Ti:Sapphire laser system ($\lambda=700$–1000 nm). A secondary electron ion microscope (SEIM) is also mounted on a pivot arm.

Fig. 2. Schematic to show the light path in a modified Nikon E600-FN research microscope for multiphoton microscopy and SHG microscopy. This arrangement depicts the off-line mode. PMT 3 is not used in the on-line mode.
sorted PMT’s placed after each optical path from the sample. With the proper light filters installed, images are acquired by correlating fluorescent emissions from a sample with the position of the scanning excitation laser.

Implementing the proposed multiphoton microscope will advance our capabilities to observe short-term kinetics of radiation response in live cells. Imaging dynamic events in 3D tissue samples will enhance our understanding of biological system response to radiation.

References


Imaging Sub-micron Particle Beams

Guy Y. Garty, Gerhard Randers-Pehrson and David J. Brenner

As we improve the spatial characteristics of the microbeam system, it becomes increasingly important to be able to assess the beam quality, in order to adjust the system to its optimum capabilities. For this purpose we are developing a secondary electron ion microscope (SEIM). This device would enable us to measure the beam profile and position, in real time, with sub-micron resolution.

The SEIM design, inspired by the common technique of photoelectron microscopy,[1] is shown in Figure 1. It is based on conversion of incident projectiles using a secondary electron emitting film, generating one (or more) electrons per projectile. The ejected electrons are then imaged, using an electrostatic unipotential lens, forming a 500 times magnified image on an image intensified CCD This high magnification enables 100 nm resolution using a 50 µm resolution CCD.

In order to overcome the chromatic and spherical aberrations inherent in the electrostatic lens, the electrons are bent by a 45° angle, reflected by an electrostatic mirror and bent by an additional 45° before reaching the detector. This “folded” design of the SEIM is a novel one, developed at RARAF.

We have built an “unfolded” SEIM, consisting of the electrostatic lens and the electron detector but without the magnet and mirror, in order to test the lens properties. For this version, simulations have shown that both the resolution and magnification are 10–20 times inferior to the folded SEIM.

For testing and calibration purposes we replaced the SEE foil with a quartz window on which a micron scale pattern of aluminum was evaporated (see Fig. 2a). The pattern was illuminated with low intensity UV light and the resulting photoelectrons were imaged, similar to a photoelectron microscope.[1] A sample image, based on ~200,000 electrons is shown in Figure 2b. The width of the spot edge allows us to estimate the SEIM resolution at 4.3 µm RMS. This is in good agreement with the simulated prediction of 4–5 µm. The predicted magnification (16x) is also in good agreement with the measured 20x.

We have performed extensive studies using SimIon (Idaho National Engineering and Environmental Laboratory – INEEL), in order to find the optimal mirror configuration for our geometry. By interfacing SimIon and Matlab (The MathWorks, Inc., MA), we have been able to implement a multidimensional search algorithm to automatically find the optimal SEIM operating parameters, for given mirror geometry.

From these systematic studies we have found the “ideal”...
Fig. 2. (a) Photo of the aluminum pattern deposited on the SEE electrode, the pitch is 200 µm. The dark spots are thick aluminum deposits having high efficiency for photoemission. (b) The pattern on (a) projected onto the MCP; the SEIM field of view for this magnification is 1 mm diameter.

SEIM configuration (a 150° mirror biased at 1.5 V with respect to the SEE film as well as the required lens voltage as a function of mirror placement), giving a single-electron resolution of 300 nm (see Fig. 3) and single electron transport efficiency of 55% (as compared to ~1% for the same resolution with a straight geometry). By raising the mirror bias to -5 V, a single electron transfer efficiency of 80% is reachable at a single-electron resolution of 500 nm. This efficiency does not include any other deficiencies in the detector system. We expect that this resolution will improve as the square root of the electron yield which is expected to be higher than unity for heavy/energetic ions, where the SEIM is needed most.

Fig. 3. (a) Simulated electron image of a 2 µm cross. The spot diameter is ~0.3 µm. This is the optimal spot in (b). (b) The dependence of the expected resolution (in microns) on the lens voltage (the acceleration voltage is 30 kV) and mirror placement (1.5 V biased 150° mirror).

References
A Microbeam Irradiator without an Accelerator

Guy Y. Garty, Gregory J. Ross, Alan W. Bigelow, Gerhard Randers-Pehrson, David J. Brenner

1. SAM – A Novel Approach
The stand-alone microbeam (SAM), under development at RARAF,[1-2] presents a novel approach to biological microbeam irradiation studies. Foregoing a conventional accelerator as a source of energetic ions, we propose to use a small, high-specific-activity, alpha emitter. This need for a SAM is motivated by the upcoming replacement of the RARAF accelerator and the need to provide an irradiation facility in the interim. The SAM is designed for simplicity and reliability of operation. It can therefore be easily reproduced in other facilities (at a small fraction of the cost of an accelerator). This would greatly facilitate research in this rapidly growing field.

2. SAM Layout
The layout of the SAM is shown in Figure 1. At the base of the SAM is a 1 mm diameter $^{210}$Po source which can be blocked or exposed, using a mechanical beam chopper. The first lens (see details below) is placed 2 m above the source, with a second (identical) lens placed 2 m above the focal plane of the first lens. At the focal plane of the first lens we place a CCD chip for measuring the beam profile. As each lens does not have identical demagnifications in the x and y axes, the two lenses are rotated by 90° in the xy plane so that a circular beam spot is obtained. A limiting aperture is placed inside the second lens to reject alpha-particles which have very large aberrations.

The cells to be irradiated are placed at the image plane. Details of the microbeam end-station are given below and in Bigelow et al.[3]

3. High Specific-Activity Isotopic Radiation Source
The radioactive source at the base of the SAM must be a monochromatic alpha emitter; with a half-life short enough to provide sufficiently high specific activity, yet long enough to allow a few months of operation between source replacements. $^{210}$Po is ideal for this purpose, as it has a half life of 138 days and decays via a single channel (a 5.407 MeV alpha-particle) into a stable daughter ($^{206}$Pb).

The dimensions of the source (1mm diameter and 200nm thick) have been chosen to optimize beam current, accounting for both the spherical and chromatic aberrations. At these dimensions spherical and chromatic aberrations are about the same.

The source will be made in-house by electroplating a 200nm thick layer of pure $^{210}$Po onto the tip of a 1mm diameter rod. Our calculations indicate we will obtain a 6.5mCi (2.4 $10^8$ DPS) source with 40keV energy spread. Assuming the alpha-particles are emitted to 4$\pi$, beam optics simulations predict a beam flux of just over 1 alpha-particle/sec at the focal plane, which is sufficient for many applications of a single-particle microbeam.

4. Ion Optics
The compound lens we will be using to focus the beam is based on the one used in the accelerator-based (electrostatic) microbeam.[4] However, in order to simplify the SAM operation, and making use of the fact that this is a fixed-energy beam that requires no daily tuning, we have elected to use permanent magnets to construct the lens. The lens can be initially tuned by extending or retracting the magnet poles (see Fig. 2), using micrometric screws and then no additional tuning is necessary.

The lack of large coils in the design allows for a smaller pole-face gap for the magnet, resulting in better focusing properties. The use of permanent magnets eliminates the need for costly power supplies and bulky cooling equipment while also significantly simplifying SAM operation. In addition, it has been shown that the spherical aberrations in a magnetic triplet are about three times lower than in an equivalent electrostatic quadrupole triplet.[5]

5. Endstation
The SAM will be mounted on an existing microbeam endstation consisting of a microscope with a particle detector mounted on the objective lens and a voice coil stage. The voice coil stage provides about 5 mm range in both x and y directions, submicron precision, better than 5 µm accuracy over 5 mm deflections and better than 0.5 µm.

Fig. 1. Layout of the SAM, see text for details.
Fig. 2. (a) Cross section of one permanent magnet quadrupole. The field intensity can be varied by extending or retracting the magnets. (b) A CAD drawing of one triplet.

accuracy over 200 µm deflections. The settling time is shorter than 200 ms. Key to the precision of a VCS is the closed-loop feedback and the response time of the overall system has been improved by using Model Predictive Control (MPC). More details are given elsewhere.\[^{[3]}\]

6. Conclusions
The SAM has been designed and is under manufacture. FEA simulations indicate that the compound lens will provide a microbeam usable for single-particle single-cell irradiations. The isotopic $^{210}$Po source will need to be replaced about once every few months. The system is designed using permanent magnets for focusing so that once settings are initially determined no further adjustment is necessary. The system uses off-the-shelf as well as custom endstation components for compact overall design.

References


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**Phase-Based Cell Imaging Techniques for Microbeam Irradiations**

*Gregory J. Ross, Alan W. Bigelow, Gerhard Randers-Pehrson, Chun C. Peng\(^1\) and David J. Brenner*

1. Introduction
Two new phase-based cell imaging techniques have been tested at RARAF for location and microbeam irradiation of cells and subcellular structures without the use of stain: 1) A novel immersion-based Mirau interferometry lens concept and 2) Quantitative Phase microscopy. Both approaches accommodate our requirement of using reflected light microscopy due to the location of the incoming ion beam. Typically, our users have used very low stain concentrations (~50 nM) as well as control studies to isolate effects from the radiation induced stresses. No-stain imaging allows increased flexibility especially in relation to future studies which will have more subtle endpoints.

Both imaging methods require the use of an automated precision vertical motion stage. In the case of Quantitative Phase microscopy, the vertical motion must be done to tens

\(^1\) Stuyvesant High School student participating in our Small Group Apprenticeship program, summer, 2004.
of nanometers of precision. And an encouraging early example of the integration of these techniques into the lab is shown in Figure 1.

2. Phase-shifting interferometry

The immersion-based Mirau interferometric objective is schematized in Figure 2. By using a high-pressure mercury lamp through a neutral cube fitted with a 540/25 filter (\(\lambda=540\) nm, \(\Delta\lambda=25\) nm), we have a coherence length of 5.1 \(\mu\)m, which aids in suppressing stray reflections and unwanted fringing.\(^{[1]}\) The beam splitter enables standard interferometry and the recombined wave contains an interference pattern which encodes the height of the sample. Three images of the sample taken at separate, pre-determined sub-wavelength distances contain enough information to solve for the modulation image such as in Figure 3.\(^{[2]}\)

As described thus far, this approach would be complicated by the thin layer of growth medium which remains over the cells under current RARAF protocols. This thin layer would cause transmission loss and therefore critical changes in relative intensity of the combining wavefronts and would also cause a change in path distance to only one of the wavefronts. Our solution is to increase the amount of medium which remains so that it reaches an immersion lens filled with the same medium, thus restoring identical wavefront pathways.

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**Fig. 1.** Example of QPm algorithm applied to normal human dermal fibroblasts. The top half is taken with the 540 nm line of the mercury lamp, in focus, which gets used along with two slightly defocused images to create the final image shown in the bottom half.

**Fig. 2.** Schematic showing the plans for an immersion-based Mirau interferometer which is being integrated into the labs at RARAF.

**Fig. 3.** Preliminary non-immersion Mirau image taken of polystyrene beads (bottom image) as compared to the same beads under room illumination (top image). The results are encouraging and show that an immersion-based system is of definite necessity.
reference mirror was based upon three competing criteria: 1) The amount of light the back of the mirror would obscure between the source and the sample and between the light source and the reference spot mirror as well as for equivalent pathways between the sample and the objective and between the reference spot mirror and the objective.

Figure 3 is a sample of encouraging preliminary results from a non-immersion-based lens. Results from the immersion-based lens under construction are expected to be much more stark and useful for the automated imaging techniques. An automated phase-unwrapping routine will be incorporated into the software to remove the ridged appearance of some objects due to the ambiguous $n \pi$ in the phase information. It should be noted that due to the thick layer of medium left over the cells, this technique will require the LD below-dish detector which is under development at RARAF.

3. Quantitative Phase microscopy

Non-interference phase microscopy can generate phase images and phase-amplitude images using a standard microscope, with either transmitted or reflected light.\(^3\) By inspecting the intensity of three images, one taken in focus, one focusing above the focal plane and one focusing below the focal plane, the light transport equation can be solved for a phase image as well as a phase-amplitude image. Figure 1 was obtained by using this technique with reflected light and applying false color to the intensity.

Software from IATIA (Melbourne, Australia) has been integrated into RARAF. Our trials have indicated that this process is over four times as costly in computer processing time as the phase-shifting (Mirau) approach and for high throughput which is offered by RARAF\(^3\) this gives some advantage to phase-shifting interferometry (PSI) over Quantitative Phase microscopy (QPm).

4. High-precision Z-motion

Both of these techniques require rapid automated motion in X-Y for locating the cells as well as in Z for changing the focal plane. In the case of immersion-based Mirau interferometry, the precision must be on the order of tens of nanometers. Therefore, we have built and installed (see Figs. 5 and 6) a custom stage with a dc-actuator and cross-roller bearing-based “macro” stage holding an LP-200 piezo-actuated nano-positioner from Mad City Labs (Madison, WI). This allows samples to be translated with better than 10 nanometer precision in three dimensions with an operating range in X and Y of several millimeters.
5. Conclusions

PSI and QPm are nearly integrated into the available protocols at Columbia University’s RARAF and offer the biological experimenter the option of using the microbeam on individual cells and subcellular structures while not incorporating any stains or dyes. PSI is currently a faster technique and therefore may for the time being be more preferable for high cell throughput studies. Both techniques can be used without removing the medium prior to irradiation.

References


Gregory Ross, Gerhard Randers-Pehrson, and Lubomir Smilenov at the Center’s year-end holiday party.
Assessment of Low-Dose Low LET Radiation-Induced Bystander Effect in a Three-Dimensional Cell Culture Model

Rudranath Persaud, Hongning Zhou, Tom K. Hei and Eric J. Hall

The radiation-induced “Bystander effect” has been demonstrated for a variety of endpoints, using a range of rodent and human cell culture models, mainly with high LET alpha-particles. However, there is a need to ascertain whether a similar response can be observed with low LET radiation at doses relevant to environmental exposure. It is equally desirable to determine if such a response can be demonstrated in a three-dimensional culture system, modeling a normal tissue microenvironment. In the present study, a three-dimensional cell culture model comprised of human-hamster hybrid (AL) and Chinese hamster ovary (CHO) cells as multi-cellular clusters was used to investigate low LET radiation-induced bystander genotoxicity. Separation of AL and CHO cells was achieved with ~99% efficiency using a magnetic cell separation technique (MACS). Briefly, tritium (3HdTTP)-labeled CHO cells were thoroughly washed to remove excess radioactivity, subsequently mixed with AL cells in a 1:5 ratio, and centrifuged briefly to produce a spheroid of 4 x 10^6 cells. Clusters were incubated overnight, resuspended into single cell suspensions, and passed twice through MACS separation columns to produce two independent cell populations. The AL fraction was plated for a 7-day expression period and subsequently subjected to the CD59 Antibody-Complement Cell Lysis Mutation Assay. Since the separation of AL and CHO cells within the cluster may not be entirely efficient, mutant colonies were detected by implementing a centromeric probe toward the human chromosome 11 present in the hybrid AL cells.

With respect to cell lethality, there was a dose-response relationship for both cell types. The lowest dose of 0.5 µCi 3HdTTP resulted in a survival of just over 90% for both cell populations, whereas the survival at the highest dose of 5 µCi 3HdTTP was 60% for CHO cells and 70% for AL cells (Fig. 1). Such results indicate a significant bystander effect induced by irradiation of neighboring cells with low LET electrons. There was also a dose-response relationship for the mutation analysis. The lowest dose of 0.5 µCi 3HdTTP produced 16 mutants/10^5 survivors that was twice the background level of 8 mutants/10^5 survivors, whereas the highest dose of 5 µCi 3HdTTP yielded 38 mutants/10^5 survivors, five times that of background (Fig. 2). Mutant spectrum analysis is currently underway to determine the types of mutation induced by such low doses of low LET radiation.

Results of the present study should provide important information on the relevance of the bystander effect under in vivo conditions. Furthermore, the mechanism(s) underlying the bystander effect is likely to be complex and may involve both primary and secondary signaling events. A better understanding of the biochemical and molecular changes governing the bystander process will help in radiation risk assessment and management.
Evidence obtained in recent years have demonstrated a biological phenomenon termed “Bystander Effect” (BE) wherein cells directly targeted transmit the damaging signal to the non-targeted cells and thereby inducing a response similar to that of targeted cells. However, the mechanism of bystander response remains largely unknown.\textsuperscript{1-2}

Protein kinase-C (PKC) is a family of serine/threonine kinases involved in the transduction of cellular signals for cell proliferation and differentiation.\textsuperscript{3} PKC, a calcium-dependent and phospholipid dependent protein, is activated \textit{in vitro} by tumor promoting agents and the data suggest that PKC plays a key role in signal transductions and various aspects of neoplastic transformation like tumor promotion and progression. The PKC family consists of 12 isozymes that have a concerted role in cell growth and differentiation. Our earlier unpublished study detected an increased clonogenic survival and growth in bystander cells treated with irradiated conditioned medium (ICM). In order to determine whether the proliferation advantage observed earlier in bystander cells is due to the PKC activation, we have analyzed the activation of PKC isoforms in irradiated and bystander cells.

Normal human fetal lung fibroblasts (MRC-5) derived from a healthy individual were obtained from Coriell Cell Repository, Camden, New Jersey. Cells were routinely maintained in 2X Eagle’s minimal essential medium (E-MEM) supplemented with 15% fetal bovine serum (Gibco BRL), vitamins, essential amino acids, non-essential amino acids and antibiotics. The cultures were maintained at 37°C in a humidified 5% CO\textsubscript{2} atmosphere. MRC-5 cells, synchronized at G\textsubscript{0}/G\textsubscript{1} phase by growing them to confluence and maintained in this state for a week with a change of medium every 3 days, were irradiated with 10 Gy of γ-rays using a \textsuperscript{137}Cs source at a dose rate of 0.98 Gy/min (Gamma cell 40, Atomic Energy of Canada, Canada). The irradiated MRC-5 cells were incubated for 1 hr at 37°C, and the medium collected from the irradiated cells (designated as irradiated conditioned medium, ICM) was used to treat the unirradiated cells for the determination of bystander effects. For sham-treated control cells, medium alone (without cells) was irradiated and used to treat the MRC-5 cells. Soluble and insoluble proteins were isolated following the protocol of Prosperi \textit{et al.}\textsuperscript{4} At different post-incubation times (0.5h, 2h, 4h and 8h), treated and mock treated cells (2h) were lysed for 10 min on ice in 300 μl of buffer-I (soluble) containing 10mM Tris-HCl pH7.4, 2.5 mM MgCl\textsubscript{2}, 1 mM PMSF and 0.5% Nonidet P-40. The cell lysates were centrifuged at 4000 rpm for 5 min and the soluble proteins (supernatant) were transferred to a fresh eppendorf tube. The pellet fraction containing the detergent of insoluble (buffer II) proteins was lysed 20 min in ice with 200 μl of 20mM sodium phosphate buffer (pH 8.0) containing 0.5 M NaCl, 1 mM EDTA, 0.75% Triton X-100, 10% glycerol, 5 mM MgCl\textsubscript{2} and 1 mM PMSF. The proteins were recovered by centrifugation at 12,000 rpm for 10 min. Protein concentration was determined by Pierce protein assay kit. Aliquots of 50–80 μg of soluble and insoluble proteins were fractionated by 4–20% polyacrylamide gradient gel electrophoresis and blotted onto PVDF membranes following the standard protocol (Invitrogen). The membranes were immersed in TBST (20 mM Tris-HCl, pH 7.4, 137 mM NaCl, 0.2% Tween) buffer containing 5% NFDM for 1h at room temperature. The membranes were incubated with primary antibodies (Cell Signal-\textit{ing Technology), to detect the endogenous and induced levels of phosphorylated PKC isoforms (PKC-βII, PKC-α/β and PKC-θ, respectively) at a 1:1000 dilution in TBST-5% NFDM for overnight and washed three times (5 min each) in TBST buffer. The membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Vector Laboratories, 1:2000 dilution in TBST-5% NFDM) for 1h followed by three times (5 min each) washing in TBST buffer. The signal was detected with enhanced chemiluminescence (ECL Kit; Amersham) upon exposure to x-ray film. The band intensity was measured using Kodak 1D image analysis software.

We have investigated the induction of phosphorylated forms of PKC-βII, PKC-α/β and PKC-θ in irradiated (10 Gy of γ-rays) and bystander cells of primary human fibroblast (MRC-5) cells at different time intervals. Western blot analysis of PKC isoforms in both irradiated and bystander cells (treated with medium harvested from 10 Gy irradiated cells) showed a noticeable induction of phosphorylated PKC isoforms at all the time points in both the cellular fractions of proteins extracted using low (soluble-buffer I) and high-salt (insoluble-BufferII) buffers. Among the PKC isoforms, phosphorylated levels of PKC-α/β and PKC-θ showed a 1.3 to 1.6 fold increase over control level in both soluble and insoluble fractions of directly irradiated cells (Fig. 1). When compared to directly irradiated cells, bystander cells showed a much higher induction of PKC-α/β and PKC-θ to 1.5–2.5 fold increase over control in both the cellular protein fractions (Fig. 1). A much higher induction of phosphorylated PKC isoforms in bystander cells compared to irradiated cells indicates that the signal for triggering PKC activation may be magnified in bystander cells. This is the first report describing the PKC activation in radiation induced bystander response. Although the mechanism underlying the transmission of damaging effects and the nature of cellular damage leading to the bystander response are not clear, we have convincingly demonstrated the activation of PKC in by-
Bystander cells, which is presumably one of the earliest events in the signaling cascade for diverse cellular responses such as secretion and gene expression. Further experiments are in progress to determine the molecular signal for PKC activation in bystander cells.

References


Cytoplasmic Irradiation Induced Bystander Mutagenesis in Mammalian Cells

Hongning Zhou, Joseph Gillispie and Tom K. Hei

In the past decade, increasing evidence has indicated that direct nucleus irradiation is not essential to induce genotoxic responses such as chromosome damage and mutation.\[1-2\] For example, irradiation of just the cellular cytoplasm could induce mutation in the nucleus of the target cells by a process involving oxiradicals.\[3\] Furthermore, very low doses of α-particles could induce a bystander response in non-irradiated bystander cells using different end points and various types of cells.\[4-5\] Using the Columbia University charged particle microbeam and the highly sensitive A\(_{L}\) cell mutagenic assay, we reported previously that cells lethally irradiated with alpha-particles could induce mutations in neighboring cells not directly hit by the particles, and that reactive oxygen species were not directly involved.\[6\] These observations have been extended to cells traversed by a single alpha-particle, and it was seen that gap junction mediated cell-cell communication played an important role in the process of bystander mutagenesis.\[7\] More recently, researchers from Gray Cancer Institute showed that cytoplasmic irradiation could induce bystander responses using a micronuclei assay as the endpoint.\[8\]

Using the Columbia University charged particle microbeam, we targeted only the cytoplasm of A\(_{L}\) cells and irradiated each cell with an exact number of alpha-particles. After irradiation, cells were maintained in the same microbeam dish overnight before being detached for further analysis. As shown in Figure 1, when 20% of the cells were irradiated with 8 alpha-particles through the cytoplasm, the induced mutant yield was significantly higher than that when assuming there was no crosstalk between the irradiated and non-irradiated cells, and the mutant yield was almost the same as that when all the cells were irradiated. Further experiments are on going to examine the mechanisms involved in cytoplasmic irradiation induced bystander mutagenesis.

To further demonstrate the bystander genotoxic effect observed with the A\(_{L}\) cells, normal human bronchial epithelial cells were used as well in this study. Using Calyculin A induced the G2 premature chromosome condensation (G2 PCC) assay,\[9]\) the preliminary data show that the incidence of chromatid-type breaks in these cells where 8 alpha-particles were delivered to the cytoplasm in 10% of the cells was significantly increased compared with those assuming no bystander effect, and the profile of chromatid breaks was very different from that in which 100% of the cells in the population were hit. These data should have high impact on radiation risk estimation, since there is a greater probability of the bronchial or lung cells to get an alpha-particle traversal through the cytoplasm than the nucleus during environment exposure.

\[References\]

Considerable evidence is now emerging that targeted nuclei may not always be required in mediating the genotoxic effects of radiation. Non-irradiated bystander cells have been shown to present similar cytotoxic and genotoxic responses to those detected in directly irradiated cells.\cite{1,2} There is evidence that gap junction mediated cell-cell communication plays a critical role in the bystander response, and that secretion of cytokines or other growth promoting factors by irradiated cells have been suggested to modulate the bystander response. However, the precise mechanism of the bystander effect is not clear. It is likely that multiple signaling pathways are required to mediate the bystander response in either confluent or sparsely populated cultures.

One of the most damaging lesions that can occur in a cell is a DNA double-strand break (DSB). The major pathway in mammalian cells dedicated to the repair of DSBs is non-homologous end-joining (NHEJ). Among the six components that play a role in NHEJ, the DNA-PK catalytic subunit (DNA-PK) complex, made up of the Ku heterodimer and DNA-PKcs, is fundamental in the first steps of the process. Mutant cells deficient in one of the DNA-PK complex components are defective in DSB repair and in V(D)J recombination and are hypersensitive to DSB inducing agents such as radiation and many carcinogenic chemicals.

In the present study, a DNA-PK deficient hamster cell line (V3) and its parental cell line (AA8) were used to study the function of DNA-PKcs in radiation-induced the bystander effect. To approach this goal, newly designed strip dishes were used in the experiments. Briefly, the bottom of the well-fit outer and inner stainless rings was covered with 6 \( \mu \)m and 38 \( \mu \)m thick mylar sheets, respectively. The mylar of the inner rings was cut as strips. Exponentially growing AA8 or V3 cells were plated in specially designed mylar dishes and cultured for several days to ensure confluency upon subsequent irradiation with alpha-particles. Cells were incubated in the mylar dishes overnight before experiments for survival, and mutagenesis were conducted. To further explore the radiation induced bystander effect in different cell lines, the charged particle microbeam was employed to lethally irradiate one type of cell in the confluent population, and the other remaining cell line was incubated for further experiments.

The AA8 and V3 cells showed similar growth kinetics as
demonstrated by growth curve analysis. However, V3 cells were more sensitive to the cell killing effect of gamma-ray irradiation. With further increase in dose of gamma rays, the \textit{HGPRT} mutation frequency decreased, likely due to the significant cell killing effect (data not shown). Our preliminary data from strip dish studies showed that alpha-particle irradiation could induce little, if any, bystander mutagenesis in DNA-PKcs deficient V3 cells, in contrast to their parental AA8 cells (Fig. 1). Using the microbeam irradiation, we found that when 10% of AA8 cells of the population get lethally irradiation, the \textit{HGPRT} mutation frequency was about 4 times higher than the spontaneous yield. However, when 10% of V3 cell in the mixed population (90% AA8 and 10% V3) were irradiated, there was only a limited bystander mutagenesis response in AA8 cells when compared with irradiated AA8 cell (Fig. 2). These data give some clue on the function of DNA-PKcs in the radiation induced bystander effect. Further experiments are needed to confirm the present finding and determine the possible signaling transduction pathways involved with DNA-PKcs in the radiation induced bystander response.

References


Separation of a Mixture of A\textsubscript{L} and CHO Cells by a Magnetic Technique and Confirmation of Their Purity by Flow Cytometry

Rudranath Persaud, Hongning Zhou, Tom K. Hei and Eric J. Hall

Many bystander studies with low LET radiation involve the analysis of the cells as one population and not separately as directly labeled/irradiated compared to the unlabeled/non-irradiated bystander cells. This study separated and isolated directly-labeled CHO cells from neighboring bystander A\textsubscript{L} cells within a three-dimensional spheroid model. This allows for the most effective evaluation of the bystander response since the bystander A\textsubscript{L} cell population can be studied independently for cytotoxicity and mutagenesis. Tritium (\textsuperscript{3}HdTTP)-labeled CHO cells were mixed with A\textsubscript{L} cells in a 1:5 ratio and centrifuged briefly to produce a spheroid of 4 x 10\textsuperscript{6} cells. Clusters were maintained at 37°C for 24 h to allow self-irradiation of CHO cells and possible traversal of any bystander signals to neighboring A\textsubscript{L} cells. The cell mixtures were treated for 30 min. at 4°C with a primary CD59 antibody that binds the cell surface antigen on A\textsubscript{L} cells. Magnetic beads, coated with FITC-conjugated rabbit anti-mouse IgG that act as secondary antibodies to the CD59 antibody, were incorporated into the cell mixtures and incubated at 4°C for 15 min. The cell mixtures were then passed twice through separation columns between magnets.

As shown in Figure 1, the effluent comprised the unbound CHO fraction whereas the magnetic bead bound A\textsubscript{L} portion remained in the column attracted by the magnets. The column was then removed from between the magnets and the A\textsubscript{L} cells were flushed with the aid of a plunger. The viability of the A\textsubscript{L} cells, based on Tryptan blue stain and a clonogenic survival assay was consistently over 90%. The independent A\textsubscript{L} cell population that results from the

Fig. 1. Diagram showing the steps involved in magnetic separation of cell populations. In step 1, CD59 antibody binds to the cell surface antigen on A\textsubscript{L} cells, then FITC-conjugated magnetic beads served as secondary antibodies; in step 2, suspension is passed through column where CHO cells are collected as effluent; and in step 3, A\textsubscript{L} cells are removed from the column.
Bystander Studies

Fig. 2. Flow cytometric analysis of clusters of A\textsubscript{L}/CHO cells after magnetic separation. Each cluster comprised of 20% CHO and 80% A\textsubscript{L} cells. Panels (A) and (B) show 100% control unstained CHO and A\textsubscript{L} cells, respectively. Panel (C) shows the purity of the A\textsubscript{L} cell fraction to be 99.24% after separation from CHO cells.

Magnetic separation can be analyzed for purity using flow cytometry that specifically identifies the FITC tag. The immunophenotypical quantification of these cells was performed using a FACSCalibur flow cytometer. Results indicated that the fraction routinely consisted of greater than 99% A\textsubscript{L} cells (Fig 2).

Can Cytoplasmic Irradiation Induce Bystander Mutagenesis in Neighboring, Non-irradiated Cells?

Targeted cytoplasmic irradiation in mammalian cells was shown by Dr. Li-jun Wu to induce gene mutations in the nuclei of the hit cells in a paper published in the Proceedings of the National Academy of Sciences in 1999 (top left). Dr. Li-jun Wu completed his dissertation work in Professor Tom Hei’s laboratory and, subsequently, won the prestigious Presidential Research Award from the Chinese Academy of Sciences that same year. The article “Cytoplasmic Irradiation Induced Bystander Mutagenesis in Mammalian Cells,” by Hongning Zhou, Joseph Gillispie and Tom K. Hei, on p. 20 of this Center report shows that cytoplasmic irradiation can also induce bystander mutagenesis (top right). This work was discussed by Professor Hei during a visit to the Chinese Academy of Sciences where he met up with Dr. Wu, now a professor there, together with many of Dr. Hei’s intellectual grandchildren (bottom).
Dr. Hei’s lab focuses on molecular mechanisms of carcinogenesis by radiation, chemicals and fibers (front, l−r): Genzi Shao, Su-Xian Liu, Sarah Huang, Honing Zhou, Tom Hei; (back): Chaing Piao, Yongliang Zhao, Vladimir Ivanov, Rudranath Persaud and Joseph Gillespie.

Dr. Lieberman’s lab focuses on molecular mechanisms of radioresistance and cell cycle control (l−r): Howard Lieberman, Kevin Hopkins, Corinne Leloup, Aiping Zhu and Xiaojian Wang.
Combined Haploinsufficiency for ATM and RAD9 as a Factor in Cell Transformation, Apoptosis and DNA Lesion Repair Dynamics


In the last few years mounting evidence suggests that heterozygocity leading to haploinsufficiency for proteins involved in DNA repair pathways plays a role in genomic instability and carcinogenesis. Haploinsufficiency for p53, PTEN, BubR1, NBS1, H2AX, p18(INK4c), BLM, Rb, APS and ATM has been shown to be an important factor in carcinogen induced tumors. Most of these genes code for tumor suppressor proteins. A major conclusion from these data is that, contrary to one of the current views on tumorigenesis, inactivation of one allele of a tumor suppressor gene is enough to contribute to tumor progression. Another conclusion from most of the cases is that animals or cells haploinsufficient for the specified proteins have higher transformation rates after DNA damage is induced, but when their DNA is not significantly damaged by exogenous sources, tumor development rates are the same as for their wild type counterparts. These data as well as the fact that most mice heterozygous for DNA repair genes have the same life span as the wild types when not challenged with mutagens, strongly suggest that haploinsufficiency is a critical factor in the cellular response to stress conditions, and even more importantly, individuals with different genotypes respond differently to the same environmental challenges.

In the case of heterozygocity when one allele of a gene is inactivated, predisposition to transformation is based on a more probable frequency of mutations than on the complete inactivation of both alleles of cancer related genes such as RB1, p53, BRCA1 or BRCA2. Epidemiological studies indicate that only 15–20% of familial breast cancer cases, for example, are a result of mutation in BRCA1 or BRCA2. The rest are most probably due to genetic factors unlikely to involve a mutation in a highly related tumorigenesis gene. Evidence suggests that the risk might be based on the additive contribution of several factors, each individually having a small effect difficult to be determined when present alone.

We hypothesize that predisposition to cancer could be a result of the additive effect of heterozygocity for two or more genes, critical for pathways that control DNA damage signaling, repair or apoptosis. Since in many cases heterozygocity leads to haploinsufficiency, we suggest that the function of signaling networks that impact on maintaining genomic integrity depends on the proper amounts of key proteins and that haploinsufficiency can lead to conditions where network efficiency under stress is suboptimal. This might result in a decrease in the effectiveness of processes related to suppression of tumor initiation, such as apoptosis and the efficient processing of DNA damage. To address this issue, cells haploinsufficient for one or two proteins related to the cellular response to DNA damage – ATM and RAD9, were examined. Both proteins are important factors in DNA double strand break repair; they rapidly colocalize to regions containing DNA double strand breaks after DNA damage, and ATM can phosphorylate RAD9. We generated mice haploinsufficient for each or both proteins, and analyzed isolated mouse embryo fibroblasts (MEFs) and thymocytes to monitor three endpoints related to tumorigenesis – cell transformation, apoptosis and DNA double strand break repair. The results demonstrate that cells having lower amounts of both ATM and MRAD9 are more sensitive to transformation induced by radiation, have different dynamics of DNA double strand break repair, retain more double strand breaks after radiation exposure and are less apoptotic than the wild type control or cells haploinsufficient for only one of these proteins. Our conclusions are that, under stress conditions, the efficiency and capacity for DNA repair mediated by the ATM/RAD9 cell signaling network depends on the expression levels of both proteins and that, in general, DNA repair network efficiencies are genotype dependent and can vary within a specific range. These findings point to the possibility of estimating an individual’s susceptibility to health risks associated with carcinogen exposure based on genotype and levels of specific proteins.

Results

Cell transformation assay:

Radiation-induced transformation of MEFs was examined to begin to access the impact of genotype on this endpoint. A total of 21 embryos from five litters were used and included five for genotypes Atmwt/Mrad9wt, Atmhz/Mrad9wt and Atmwt/Mrad9hz and six for Atmhz/Mrad9hz. Yields of transformed clones were measured both for unexposed controls and after a dose of 2 Gy. The results shown on Table 1 indicate a statistically significant higher transformation frequency for the double heterozygous cells. Transformation frequencies for these cells are more than double that of the wild type population. The Mrad9 heterozygous cells demonstrate a transformation frequency close to that of the wild type cells, and the frequency for the ATM heterozygous cells is between the wild type and double heterozygous cells.

Apoptosis of thymocytes:

We examined thymocytes from single and double heterozygous animals for radiation induced apoptosis. The number of animals and the genotypes used were respec-
The differences between apoptotic frequencies in after irradiation, while ATM deficient cells show the lowest. 1). Wild type cells display the highest apoptotic frequencies differences in apoptotic frequencies related to genotype (Fig. mice were from 4 different litters. The results demonstrate type and het erozygous cells were small but statistically sig- tively: Atmwt/Rad9wt - 5, Atmhz/Rad9hz - 7, Atmwt/ Rad9hz - 5, Atmhz/Rad9wt - 6, Atmko/Rad9wt - 3. The mice were from 4 different litters. The results demonstrate differences in apoptotic frequencies related to genotype (Fig. 1). Wild type cells display the highest apoptotic frequencies after irradiation, while ATM deficient cells show the lowest. The differences between apoptotic frequencies in Atm wild type and heterozygous cells were small but statistically signif- icant and show that Atm heterozygocity could be a factor influencing programmed cell death. Mrad9 heterozygous cells show the same apoptotic rates as the wild type control. Remarkably, the apoptotic frequencies were significantly reduced in the double heterozygous cells. The results for this genotype are closer to those obtained for the Atm null cells than for the wild type, showing that haploinsufficiency for two functionally related proteins may have an additive negative effect on pathways where both proteins are normally involved. Since ATM is an important factor in T-cell differ- entiation, thymocytes from all animals were examined for CD4/CD8 markers. No difference was found in double posi- tive and single positive distributions except for the ATM deficient thymocytes where, as expected, a partial block at the CD4/CD8 double positive stage and reduced numbers of single positive CD4 and CD8 cells were found (data not shown).

DNA double strand break repair dynamics:

The appearance of DNA double strand breaks and their repair in MEFs having different genotypes were examined. Cells were passaged until close to senescence since fast proliferating early passage MEFs show high background γ-H2AX staining. Changes in the number of foci formed in response to 0.5 Gy of γ-rays were followed for up to 24 hours (Fig. 2). The genotypes evaluated were Atmwt/Mrad9wt, Atmwt/Mrad9hz, Atmhz/Mrad9wt, Atmhz/Mrad9hz, Atmko/Mrad9wt. There was no significant difference between the number of foci in the wild type and single heterozygous cells. γ-H2AX foci formation was slower in the Atmhz/Mrad9hz cells but after 2 hours were statistically equal to those demonstrated by cells with the other genotypes. After 24 hrs the Atmhz/Mrad9hz cells show more residual dsb than wild type and the single heterozygous cells. The Atm knockout cells had the highest background staining and showed slow foci formation, and high residual foci frequencies after 24 hours.

**Table I.**

<table>
<thead>
<tr>
<th></th>
<th>Atmhz/</th>
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<tr>
<td></td>
<td>Mrad9wt</td>
<td>Mrad9hz</td>
<td>Mrad9hz</td>
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<tr>
<td>Relative</td>
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<td>1.45</td>
<td>3.10</td>
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![Fig. 1. Apoptosis of thymocytes having different genetic backgrounds. Thymocytes from mice were irradiated with different doses of γ-rays and apoptosis was measured 6 hrs after irradiation. The percent apoptotic cells at 0 Gy was subtracted from the rest of the data points for each genotype.](image1)

![Fig. 2. Double strand break repair in different genetic backgrounds. MEFs having the indicated genotypes were irradiated with 0.5 Gy of γ-rays. The number of DNA double strand breaks was revealed by staining with anti γ-H2AX anti-](image2)
Discussion

Haploinsufficiency as a result of heterozygocity for tumor suppressor genes in combination with carcinogens has been implicated in tumorigenesis. Our previous results show that heterozygocity for Atm increased modestly the transformation of MEFs after irradiation, and was also a factor in cataract formation. Haploinsufficiency for Atm has long been suspected as a contributing factor in familial breast cancer as well as other types of cancer. Theoretically, all familial cases of tumor development where one protein is haploinsufficient or deficient, points to other important factors characteristic for the family. Since usually no mutations in other genes have been identified in many of these cases, we hypothesize that some of these unknown factors could be a second haploinsufficiency for a protein related functionally to the first one. In this respect, we analyzed three cancer related events: cell transformation, apoptosis and double strand break repair in cells single or double haploinsufficient for Atm and Mrad9. We found that haploinsufficiency for both proteins had an additive effect, noticeably increasing cell transformation after radiation induced DNA damage, and decreasing apoptotic frequencies in irradiated thymocytes, bringing the apoptotic levels closer to that observed in the Atm null phenotype. Additionally, the double haploinsufficient phenotype changed the dynamics of dsb repair and decreased the efficiency of removal of those lesions, as shown by staining for γ-H2AX. These data are consistent with a model indicating that low levels of ATM and MRAD9 proteins result in a less efficient cellular response to excessive DNA damage, including DNA repair and apoptosis.

These results indicate that haploinsufficiency for two closely interacting proteins (i.e., ATM and MRAD9) functioning in related pathways could play a significant role in tumorigenesis by altering the mechanisms preventing it. Another conclusion is that the effectiveness of DNA repair networks may vary within a specific range depending on the genotype and levels of the proteins involved. An additive effect of a second haploinsufficiency may significantly contribute to the destabilization of a particular cell signaling network. Therefore, it appears that cell signaling networks are finely tuned and are most effective only when the concentration and consequently the activity of the proteins involved are at optimal levels. As a result, the capacity for DNA repair under stress conditions will depend on an individual’s genotype, and the degree of accumulation of mutations in the presence of the same dose of mutagens could vary from individual to individual.

Interpretation of these findings in the context of tumor initiation and progression leads to the suggestion that initial events in transformation could arise in a heterozygous background of partially destabilized networks and the presence of mutations. Mutations where one copy of a gene is inactivated are much more probable than complete gene inactivation. Therefore, the additive effect of two or more haploinsufficiencies may be a decisive factor in the initial accumulation of damage leading to cancer.

Currently there are two basic paradigms for the initial stage of tumorigenesis – mutator phenotype and aneuploidy. According to the mutator phenotype hypothesis, the initial stages of carcinogenesis are a result of mutation of genetic stability genes which increase mutation rates for other genes, and eventually lead to cell transformation. The aneuploidy model suggests that mutation of a small number of genes required for cell division leads to chromosome breaks or unequal chromosome segregation. This results in genetic instability and the generation of mutations in multiple genes. The aneuploidy model explains well how small numbers of initial mutations could lead to the high subsequent number of mutations needed for cancer progression. A problem with both models is the inability to explain how the initial mutations occur since efficiency of DNA repair networks in normal cells is very high and the probability of complete inactivation of even 3–5 relevant genes in the course of the lifetime of a cell is very low. Our results suggest that DNA repair pathway effectiveness could be significantly lowered by a few mutations affecting one copy of related genes. This, especially when combined with the presence of environmental mutagens, may result in a high number of DNA lesions, mutation accumulation and tumor initiation. This model could support the conclusion that each individual has unique inherent sensitivity to mutagens, depending on the levels or activity of the proteins involved in DNA repair or related processes. This sensitivity could be measured in vitro by the analysis of genotype and levels of specific proteins. This could lead to the establishment of individual limits for mutagen exposure that will bear a health risk, and may thus have significant consequences for cancer prevention.

Acknowledgments
We thank Dr. Olga Sedelnikova and Dr. W. Bonner, NCI, NIH, for providing us with the γ-H2AX antibody and for the methodological help regarding these experiments.

References
5. Srivastava M, Montagna C, Leighton X, Glasman M, Naga S, Eidelberg O, Ried T and Pollard HB. Haploin-


Human hRAD9 is a key member of the Rad family of checkpoint proteins and is a nuclear protein. It can be phosphorylated by ATM in response to DNA damage. hRAD9 is involved in the detection of DNA damage, cell cycle checkpoint control, and DNA repair. The N-terminus of hRAD9 contains a region that is similar to a region in the proliferating cell nuclear antigen (PCNA) and associates with hRAD1 and hHUS1, forming a hRAD9-hRAD1-hHUS1 heterotrimer. This protein complex is loaded onto DNA via hRAD17 to serve as part of a DNA damage sensing mechanism. Recently, it was reported that the hRAD9 C-terminal domain contains a FXXLF motif and that it may mediate an interaction with the androgen receptor (AR) ligand binding domain. There is evidence that this hRAD9-AR binding can suppress the androgen-induced interaction between the N-terminus and C-terminus of AR and result in repression of the ability of AR to transactivate a series of downstream target genes important for prostate function.

In order to learn more about the hRAD9 association with prostate cancer, co-immunoprecipitation was performed. Prostate cancer cell lines LNCaP and PC-3 were used. Both cell lines express endogenous hRAD9. LNCaP contains androgen receptor, whereas PC-3 does not (used as negative control). Confluent (80%) LNCaP and PC-3 cells were exposed to gamma rays in 75 cm² flasks. After 4 and 8 hours post-irradiation, cells were lysed in 1 ml of ice-cold cell buffer. Primary antibody directed against hAR was added to the precleared whole cell lysate and the cell lysate was incubated overnight at 4°C. Anti-hRAD9 antibody detected hRAD9 via western blotting. The results showed that endogenous hRAD9 could bind with hAR in LNCaP prostate cancer cells only after the cells were exposed to gamma rays. The binding was a little stronger at the 10 Gy dose than it was at 2 Gy. There was no binding in the PC-3 cell line, which has no AR (Fig. 1). These results suggest that hRAD9 and AR can physically interact with each other under the present test conditions only after radiation. Their binding may interrupt AR-androgen interaction and further suppress transcription of downstream genes (Fig. 2).

Some clinical data have shown that eliminating androgen improves the survival of patients with locally advanced prostate cancer when combined with radiation therapy. A few prostate cancer patients’ samples showed that expression of hRAD9 is reduced in prostate tumors compared to normal prostate tissues. This suggested that deregulated expression of hRAD9 may be involved in the progression of prostate cancer.

Fig. 1. Immunoprecipitation of endogenous AR and hRAD9 in LNCaP prostate cancer cell line. (A) Immunoprecipitation (IP) was performed with antibody against AR, followed by immunoblotting with antibody against hRAD9. A strong co-IP was detected in LNCaP AR (+) cells after irradiation. There was no co-IP detected in PC-3 AR (-) cells (as a negative control), and even LNCaP AR (+) when cells were not first irradiated. (B, C, and D) Western-blotting: (B) AR protein was detected in LNCaP cells, there was no expression of AR protein in PC-3 cells by anti-hAR antibody. (C) hRAD9 protein was detected in LNCaP and PC-3 cells by anti-hRAD9 antibody. (D) The total amount of sample loaded per well is very similar, as judged by probing for β-actin.

Fig. 2. Model of hRAD9, AR and Androgen interactions.
cancer. Loss of hRAD9 expression in cells may impair checkpoint activation, reduce DNA repair, and increase cell proliferation mediated by androgen or AR.[4] Our data and work by other investigators showed that hRAD9 likely plays an important role in prostate function and in prostate cancer. Nevertheless, additional studies are needed to define that role in more detail, as well as to exploit this finding to gain therapeutic benefits.

References


DUSP1 Is an Essential Target of E2F-1 in the Apoptotic Response to Oxidative Stress

Jianli Wang and Yuxin Yin

The E2F family includes six distinct, but closely related transcription factors that regulate cell proliferation, development or apoptosis. The best known member of the E2F family is E2F-1.[1] E2F-1 binds to variants of the consensus sequence.[2–3] This consensus sequence is found in the promoters of a number of genes important for cell cycle progression, including c-myc, cdc2, cdc6, dihydrofolate reductase, thymidine kinase and the E2F-1 gene itself.[4–5] E2F-1 plays an important role in cell cycle regulation. It promotes entry into S phase and stimulates cell cycle progression.[6] E2F-1 function is negatively regulated by Rb, which binds to the transactivation domain of E2F-1 and suppresses E2F1-mediated transcriptional activation.[7–8] E2F-1 acts as a cell death mediator in certain settings and functions as a tumor suppressor in a tissue specific manner.[9–10] Overexpression of E2F-1 can trigger apoptosis,[11–12] indicating that E2F-1 is involved in cell death control. E2F-1 was initially considered to be an oncogene until 1996 when it was shown that mice lacking E2F-1 develop a broad spectrum of tumors.[10] Also, E2F-1-deficient mice exhibit defects in apoptosis and aberrant cell proliferation in some tissues.[13] These findings demonstrate that E2F-1 is a tumor suppressor. The mechanisms, however, by which E2F-1 inhibits tumorigenesis are largely unknown. It is commonly believed that E2F-1 functions as a tumor suppressor through its induction of apoptosis in p53-dependent and -independent manners.[14] One of the significant targets identified so far for E2F-1 function in apoptosis is the p53 homologue p73.[15–16] E2F-1 regulates transcription of p73, which can induce apoptosis in the absence of p53.[17–18]

Using DNA microarray technology, we observed that DUSP1 is a potential target of E2F-1. DUSP1 was originally cloned as CL100 because of its response to oxidative stress[19] and it functions to dephosphorylate and inactivate MAP kinases.[20] To determine whether E2F-1 can induce the expression of DUSP1 in vivo, we introduced a CMV-driven E2F-1 expression vector (pCMV/E2F-1) into MCF-7, a breast cancer cell line with low levels of endogenous E2F-1 (data not shown). We obtained stable clones expressing ectopic E2F-1 through G418-selection and western analysis of E2F-1 protein (data not shown). These clones were designated as MCF7/E2F1, followed by clone numbers. If E2F-1 is a positive regulator of DUSP1, the levels of DUSP1 expression should be increased in cells expressing E2F-1. As shown in Figure 1A, the DUSP1 transcript is low in MCF/pCMV (upper panel, lane 1). However, DUSP1 mRNA is largely increased in MCF7/E2F1 clones (lane 1 vs. lane 2, 3). Correspondingly, the DUSP1 protein level is elevated in the presence of ectopic E2F-1 (Fig. 1B, lane 2, 3). These results indicate that DUSP1 transcription is regulated by E2F-1.

To determine whether DUSP1 is responsive to genotoxic stress and whether its response is dependent on E2F-1, mouse embryo fibroblasts (E2F-1+/+ and E2F-1−/− MEFs) were treated with γ-irradiation, UV, serum deprivation, or H2O2, respectively. As shown in Fig. 1C, expression of DUSP1 is low in E2F-1+/− MEFs and not induced by γ-irradiation (lanes 1, 2). In addition, DUSP1 is not induced by
either serum starvation or a moderate dose of ultraviolet C-irradiation (Fig. 1C, lanes 4 and 5), which also causes cell cycle arrest [25]. However, DUSP1 levels markedly increase following oxidative damage by H₂O₂ (lane 1 vs. lane 3). Furthermore, the transcriptional response of DUSP1 to oxidative damage is completely diminished in E2F-1−/− MEFs (lane 3 vs. lane 8). These observations suggest that DUSP1 is inducible by oxidative stress in an E2F-1-dependent manner and that E2F-1 selectively regulates DUSP1 in response to oxidative damage. To determine the role of E2F-1 in oxidative cell death, we measured cell viability under oxidative stress in relation to E2F-1. As shown in Fig. 1E, MCF-7 cells were mostly killed by H₂O₂ in the presence of ectopic E2F-1 expression whereas a large number of the MCF-7 cells survived under the same conditions in the absence of E2F-1, suggesting that E2F-1 is a mediator of cell killing following oxidative stress. The importance of E2F-1 in the cellular response to oxidative stress was further confirmed in Fig. 1F where E2F-1+/+ MEFs were susceptible to H₂O₂ but E2F-1−/− MEFs were resistant to oxidative damage.

To demonstrate the importance of DUSP1 in signaling oxidative cell death, we used the pSilencer 1.0-U6 siRNA expression vector to construct a DUSP1/siRNA, which is under the control of a U6 RNA Polymerase III promoter, to knockdown DUSP1 expression. The DUSP1/siRNA vector was introduced into MEFs and the empty vector pSilencer 1.0 U6 was used as a control. Stable clones were selected by administration of hygromycin B and further tested for DUSP1 expression by Northern blotting. As shown in Figure 2A, while its expression in MEF/U6 cells is increased following H₂O₂, DUSP1 levels remain unchanged in cells expressing DUSP1 siRNA, indicating that expression of these DUSP1 siRNAs interferes with transcription of DUSP1. With these cells available, we examined the sensitivity of MEF/U6 and MEF/DUSP1/siRNA to H₂O₂ (Fig. 2B). As anticipated, cells expressing DUSP1/siRNA are resistant to oxidative damage.

**Fig. 1.** Transcriptional regulation of DUSP1 and induction of cell death by E2F-1. (A) Induction of DUSP1 transcription by ectopic E2F-1. MCF7/pcDNA3 and MCF7/E2F-1 clones were cultured under normal conditions. Total RNA was equally loaded and Northern blot analysis was performed using the indicated probes. (B) Induction of DUSP1 protein by p53 under serum starvation. Cell extracts were resolved by 10% SDS-PAGE and transferred for Western blotting using anti-DUSP1 or anti-actin antibodies. (C) Expression of DUSP1 transcription in mouse embryo fibroblasts under various conditions. Exponentially growing MEF cells were exposed to γ-irradiation (6 Gy), H₂O₂ (100 μM), serum starvation (0.1%FBS), or UV-irradiation (10 J/m²), respectively as indicated. The cells were harvested after 4 hours and RNA from each group was fractionated on a 1.2% formaldehyde agarose gel and transferred for Northern blotting using a dCTP-labeled DUSP1 cDNA probe, following standard procedures. The blots from A and C were rehybridized with GAPDH or β-actin cDNA probes as loading controls. (D) Mediation of cell death by E2F-1 following oxidative damage. The indicated cells were treated with H₂O₂ (200 μM) for 24 hours and viable cells were counted by trypan blue exclusion. (E) Requirement of E2F-1 for the cellular response to oxidative stress. Exponentially growing E2F-1+/+ MEFs and E2F-1−/− MEFs were treated with H₂O₂ (100 μM) for 24 hours and were scored for viable cells by trypan blue exclusion.
Fig. 2. Role of DUSP1 in the cellular response to oxidative damage. (A) Reduction of mouse DUSP1 expression by DUSP1siRNA. MEFs were transfected with either a control pSilencer U6 or U6/siDUSP1 vectors. Exponentially growing cells, including MEF/u6 and the cells with DUSP1 siRNA, were treated with H2O2 (100µM) for 3 hours, or not at all, and the level of DUSP1 expression was measured by Northern blot analysis. (B) Requirement of DUSP1 in the cellular response to oxidative stress. Exponentially growing MEF/u6 and MEF/DUSP1siRNA cells were treated with H2O2 (100µM) for 24 hours. Cell viability was determined by trypan blue exclusion. The results are represented as the means ± S.D. of three independent experiments of duplicate cultures. (C) Ectopic expression of DUSP1 in MCF-7 cells. MCF-7 cells were transfected with either pcDNA3 or pcDNA3/DUSP1 and selected for stable clones. Equal amounts of total RNA from MCF7/pcDNA3 and MCF7/DUSP1 cells were resolved and transferred for Northern blotting using the corresponding [α-32P]dCTP-labeled cDNA probes. (D) Cell viability with or without ectopic DUSP1 under oxidative stress. The indicated cells were treated with H2O2 (200µM) for 24 hours. The viable and nonviable cells were scored by trypan blue exclusion. (E) Anchorage-independent colony formation in soft agarose of MCF7/pcDNA3 and MCF7/DUSP1 cells. The experiments were carried out in triplicate. The values are the means ± standard deviation of two independent experiments.

oxidative stress by H2O2, whereas the majority of the control MEF/U6 cells are killed by the same dosage of H2O2. These results confirm the importance of DUSP1 in the cellular response to oxidative damage.

In order to determine whether DUSP1 mediates the cellular response to oxidative stress, we constructed a human DUSP1 expression vector by cloning a 1.4 kb human DUSP1 cDNA containing the full coding sequence in frame into a mammalian expression vector driven by a human cytomegalovirus promoter (pcDNA3/hygro, Invitrogen). The resulting DUSP1 expression vector, pcDNA3/DUSP1, or an empty vector pcDNA3, was transfected into MCF-7, a breast cancer cell line containing a low level of DUSP1, which was then challenged with hygromycin B for isolating stable clones of MCF7/DUSP1. We chose MCF7/pcDNA3 cells and MCF7/DUSP1 cells containing a high level of ectopic DUSP1 expression (Fig. 2C) for functional studies. We found that while MCF7/pcDNA3 cells are resistant to oxidative stress, these MCF7/DUSP1 cells are highly susceptible to cell killing by H2O2 treatment (Fig. 2D). These results indicate that DUSP1 functions as a cell death mediator in the cellular response to oxidative stress. To demonstrate that DUSP1 suppresses tumor formation, we performed two experiments. First, we examined the colony formation capability of MCF7/pcDNA3 and MCF7/DUSP1 cells in soft agar and then determined whether these cells formed tumors in nude mice. We found that there is much less colony formation of the MCF7/DUSP1 cells than that of control MCF7/pcDNA3 cells (Fig. 2E).

In this report, we demonstrate a new mechanism for E2F-1 to mediate cell death in response to oxidative damage. We report that E2F-1 is a transcriptional regulator of DUSP1 and that E2F-1 is required for the response of DUSP1 to oxidative stress. We further demonstrate that DUSP1 is important for E2F-1 function in signaling oxidative cell death. Although it is well known that E2F-1 functions as a tumor suppressor, the mechanism involved is largely unclear. Because DUSP1 is a cell death mediator, DUSP1 may be a downstream effector of E2F-1 functions in controlling cell fate and tumorigenesis.
Downregulation of Betaig-h3 Gene Is Involved in the Tumorigenic Process of Human Bronchial Epithelial Cells Induced By Heavy Ion Radiation

Yongliang Zhao, Genze Shao, Chang Q. Piao, Jessica Berenguer and Tom K. Hei

High energy (HZE) heavy ions, when compared to low-LET radiation, are highly effective in inducing gene mutation, chromosomal aberrations and neoplastic transformation. However, the underlying molecular mechanisms are not clearly understood. We have recently shown that the downregulation of Betaig-h3 expression is causally linked to the tumorigenic phenotype of papillomavirus-immortalized human bronchial epithelial (BEP2D) cells treated with high-LET alpha-particle radiation. Using the BEP2D cell culture system, a radiation induced transformation model has been established by a single 60 eGy dose of $^{56}$Fe heavy ion radiation.\[1\]
To check whether downregulation of the *Betaig-h3* gene is a frequent event in $^{56}$Fe radiation-induced tumorigenic BEP2D cells, Northern blot was employed to check the mRNA levels of this gene in three heavy ion-induced tumorigenic cell lines ($^{56}$FeT1-T3). As shown in Figure 1, expression of *Betaig-h3* gene was downregulated by 6–8 fold in $^{56}$FeT1-T3 cell lines when compared with parental BEP2D control cells.

The significance of *Betaig-h3* downregulation in the tumorigenic process was determined by re-expression of *Betaig-h3* in the highly tumorigenic $^{56}$FeT2 cells by using the pRc/CMV2-Betaigh3 vector. The empty pRc/CMV2 vector was used as the control. From Figure 2, the parental $^{56}$FeT2 and $^{56}$FeT2-pRc/CMV2 cells expressed a very low and similar level of *Betaig-h3* protein relative to control BEP2D cells. After *Betaig-h3* transfection, expression of this gene in clone 17 was recovered to a similar level found in control BEP2D cells, whereas clone 31 had a 2-fold higher level.

To determine if ectopic expression of *Betaig-h3* gene in heavy ion-induced tumorigenic cells could suppress the tumorigenicity in vivo, we injected $5 \times 10^6$ cells of each of the following cell lines into nude mice, including control BEP2D cells, $^{56}$FeT2 tumorigenic cells, $^{56}$FeT2-pRc/CMV2, and *Betaig-h3* transfected cells (Clone 17 and 31). The tumor volumes were measured weekly during the experiments. As shown in Table I, no tumors were formed for control BEP2D cells after more than 20 weeks. However, mice injected with $^{56}$FeT2 (8/8) and empty vector-transfected $^{56}$FeT2 cells (8/8 mice) produced progressively growing tumors at 4 weeks with the average tumor volume of 337.55 mm$^3$ and 377.39 mm$^3$. In contrast, the sites (16/16 mice) injected with *Betaig-h3* transfected cells (clone 17 and 31) formed significantly smaller tumors than parental $^{56}$FeT2 cells, with the tumor volume of 88.02 mm$^3$ ($P<0.05$). Meanwhile, at the time points of 2 and 3 weeks the tumor growth (Fig. 3) was significantly suppressed in *Betaig-h3* transfected $^{56}$FeT2 tumorigenic cells.

To determine whether downregulation of the *Betaig-h3* gene...
Fig. 4. Protein level of biologically active TGF-β1 in culture medium determined by immunoblotting. Total TGF-β1 in whole cell lysates were used as control. Human anti-active TGF-β1 (Promega) and Anti-TGF-β1 (Santa Cruz) antibodies were used in this study.

gene results from dysregulation of TGF-β1, biologically active levels of TGF-β1 were screened by using western blotting. As shown in Figure 4, nontumorigenic BEP2D cells have a relatively low level of active TGF-β1 in the culture medium. However, two out of three tumorigenic cell lines (56FeT2-T3) has 2–3 fold higher levels of active TGF-β1, except 56FeT1 has a similar level, compared with BEP2D cells. To further determine if downregulation of the Betaig-h3 gene is due to inactivation of downstream genes of TGF-β1, induction of Betaig-h3 expression was investigated by incubating the control BEP2D and malignantly transformed cells with 5 ng/ml TGF-β1 for 48 h. From Figure 5, the protein level of Betaig-h3 gene was 2-fold higher in TGF-β1 treated BEP2D cells than that in untreated cells. However, expression of this gene was slightly induced by exogenous TGF-β1 in 56FeT1-T3 cells, and much lower than control BEP2D cells. In addition, no mutations or deletions were found in the Betaig-h3 gene in 56FeT1-T3 cells by using southern blotting and cDNA sequencing techniques (data not shown).

In vitro assay systems such as oncogenic transformation have been used to examine the biological effectiveness of heavy ion radiation.\(^3\)\(^-\)\(^4\) By using a BEP2D cell model, malignantly transformed cells have been established by a single 60 cGy of 56Fe heavy ions six months after irradiation.\(^5\)\(^\)\(^6\) Transformed cells progress through sequential stages including altered growth kinetics and anchorage independent growth, before becoming tumorigenic and producing progressively growing subcutaneous tumors following inoculation into athymic nude mice. In addition, results from the fusion experiments demonstrated that the tumorigenic phenotype in BEP2D cells induced by 56Fe ions could be completely suppressed by fusion with nontumorigenic BEP2D cells. The data suggest that loss of tumor suppressor gene(s) is a likely mechanism in 56Fe carcinogenesis.\(^7\)\(^8\) Therefore, the tumorigenic BEP2D cell model is useful for studying the genetic events involved in 56Fe ion-induced tumor progression. In this study, we show here that Betaig-h3 expression is significantly suppressed in three 56Fe ion-induced and independently-generated tumorigenic cell lines (56FeT1-T3). Ectopic expression of this gene in the 56FeT2, one of the highly tumorigenic cell lines, significantly suppresses their in vivo tumorigenicity. Our finding strongly suggests that the loss of Betaig-h3 expression plays a pivotal role in heavy ion carcinogenesis.

Fig. 5. Induction of Betaig-h3 gene expression by incubating the control BEP2D and 56FeT1-T3 tumorigenic cells with 5 ng/ml TGF-β1 for 48 h. Samples containing equal amounts of proteins concentrated from the culture medium were loaded and used for immunoblotting analysis.

References


2. Zhao YL, Piao CQ and Hei TK. Overexpression of Betaig-h3 gene downregulates integrin alpha5 beta1 and suppresses tumorigenicity in radiation-induced tumorigenic human bronchial epithelial cells. \emph{Br J Cancer} \textbf{86}:1923-8, 2002.


Profilin of Differentially Expressed Genes Induced by Organophosphorous Pesticides and Estrogen of Human Breast Epithelial Cells

Gloria M. Calaf,1 Debasish Roy2 and Tom K. Hei

Environmental chemicals may be involved in the etiology of breast cancer.1 Organophosphorous compounds are the most widely used pesticides by virtue of their biodegradable nature and short persistence. Such compounds are of great interest because of the extensive use in agriculture, medicine and industry. Breast cancer risk is associated with prolonged exposure to female hormones. Among the various hormones of interest, a leading role is attributed to estradiol, since breast cancer does not develop in the absence of ovaries. Methods to define patterns of gene expression have applications in a wide range of biological systems. Several molecular biological techniques are used to study gene expression patterns during the neoplastic progression of breast epithelial cells. The aim of the present study was to identify the differentially expressed human cell cycle genes that were altered by organophosphorous pesticides and estrogen in human breast epithelial cell lines using cDNA arrays.

MCF-10F, an immortalized human breast epithelial cell line was treated with the organophosphorous pesticide, parathion, either alone or in combination with estrogen. Malignant cell lines developed through a series of sequential steps. Previously studies have showed that parathion and 17 beta estradiol induced malignant transformation of MCF-10F as indicated by increased cell proliferation, invasive capabilities and increased PCNA, mutant p53, beta catenin and RhoA protein expression in comparison to control MCF-10F cells (Table I).2 Expression of the cell cycle genes in the parental MCF-10F and in the transformed cells induced by the various treatments were ascertained using the 96 gene human cell cycle gene array (from Super Array, Biotechnology Corporation, MD).3 In mammalian cells, cell cycle progression is precisely controlled by cyclin-dependent kinases (CDKs) and proteins that regulate CDKs. These CDKs and CDK-modifying proteins, including cyclins, CDK inhibitors, CDK phosphatases, and CDK kinases, are featured in the Human Cell Cycle GE Array Q Series. Genes essential for DNA damage and mitotic spindle checkpoints, as well as genes in APC ubiquitin-conjugation complexes, are also represented.4–12 By using the experimental RNA samples of MCF-10F cells treated with estrogen, parathion and parathion plus estrogen in comparison to control MCF-10F the array was able to simultaneously quantify and analyze the expression profile of 96 genes involved in cell cycle regulation.

Results indicated that among the various genes that regulate the human cell cycle, 19–22 were found to be altered either by estrogen alone, parathion or a combination of both (Fig. 1). The altered genes included those that related to S phase as cyclins (A1, A2, C, G1, G2, H); CDKs;13–15 and minichromosome maintenance deficient (MCM). Among them, cyclin D3 gene expression was increased 2–5 fold in the estrogen and parathion-treated group in comparison to control. MCM6 gene expression was increased 2–5 fold in the estrogen and parathion plus estrogen-treated cells (Table II). The MCM6 labeling index has been previously correlated with proliferative activity and tumor grade in chondrosarcomas.16 It can be concluded that pesticides in the presence of estradiol were capable of inducing transformation of human breast epithelial cells and altered cell cycle gene expression. These studies suggest that organophosphorous pesticides and estradiol induced changes in gene ex-

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<tr>
<td>Parathion + E</td>
</tr>
</tbody>
</table>

Anchorage Independence: colony-forming efficiency in agar fluctuated from 1–3%.
Invasion: invasive characteristics of control and irradiated MCF-10F cells scored 20 h after plating onto matrigel basement membranes using modified Boyden’s chambers constructed with multi-well cell culture plates and cell culture inserts.
Tumorigenicity: tumors formed in nude mice. Average of 6 animals/group.
Positive signs (+): represent the results in relation to anchorage independent growth and number of cells that crossed the filters.
Negative signs (-): lack of anchorage independent growth, invasiveness or formation of tumors in the nude mice.
E: 17 β estradiol (10-8 M).
ND: non-determined.

1 University of Tarapaca and Research Center for the Man in the Desert, Arica, Chile.
2 Brookhaven National Laboratory, Biology Department, Upton, New York.
Differentially expressed genes in estrogen and parathion-treated cells

<table>
<thead>
<tr>
<th>Array Location</th>
<th>Gene Name (Symbol)</th>
<th>MCF-10F + Estrogen</th>
<th>MCF-10F + Parathion</th>
<th>MCF-10F + Parathion + Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>G1/S-specific Cyclin C (CCNC)</td>
<td>--</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Cyclin D3 (CCND3)</td>
<td>--</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>29</td>
<td>Cell division cycle 6 (CDC6)</td>
<td>--</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td>Cyclin-dep kinase inhibitor 1A (CDKN1A, p21Waf1)</td>
<td>--</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>41</td>
<td>Cyclin-dep kinase inhibitor 2C (CDKN2C, p18, cdk4 inhibitor)</td>
<td>--</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>44</td>
<td>CDC28 protein kinase 1 (CKS1)</td>
<td>--</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>69</td>
<td>Mouse double min 2, Hum homolog of p53 bind protein (MDM2)</td>
<td>--</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>67</td>
<td>Minichromosome maintenance deficient 6 (MCM6)</td>
<td>--</td>
<td>↑</td>
<td>-</td>
</tr>
</tbody>
</table>

(↑) Indicates the up-regulated expression of that gene with respect to control MCF-10F.
(↓) Indicates the down-regulated expression of that gene with respect to control MCF-10F.
(-) Indicates no change in expression of that gene with respect to control.

Note: Both up-regulation and down-regulation of gene expression over 2–5 fold alterations are taken into consideration.

Fig. 1. Expression of cell cycle genes in the parental MCF-10F (top left), MCF-10F + Estrogen (top right), MCF-10F + Parathion (bottom left) and MCF-10F + Parathion + Estrogen (bottom right) cell lines.

Expression in the human breast epithelium influencing the carcinogenesis process.

References

Gene Expression in Response to Heavy Metal Stress

Sally A. Amundson, Khanh T. Do\(^1\) and Albert J. Fornace Jr.\(^1\)

Heavy metals, such as cadmium and arsenic, are known to be both toxic and carcinogenic. They are also widespread environmental contaminants, posing a serious threat to human health in many parts of the world. They cause cellular damage largely through the generation of oxidative stress, although the exact mechanisms of toxicity and carcinogenesis are not yet understood. As has been observed for most toxic stresses, heavy metal exposure can affect gene expression, through modulation of NF-kB, AP-1 and p53 activities.

As part of an effort to characterize molecular responses to diverse stressors, we have exposed two human cell lines (TK6, a p53 wild-type cell line, and NH32, its p53-null derivative) to a panel of 12 different agents, including sodium arsenite. A 4-hour exposure to graded doses of arsenite resulted in increased apoptosis after 24 hours, with only a slight dependence on p53 function (Fig. 1A). Similarly, the effect of p53 status on the response of several commonly induced genes at the end of the 4-hour exposure period (Figure 1B) was much less than that seen for agents such as ionizing radiation.

Microarray analysis was used to compare gene expression in these cell lines following metal and other stress exposures. Stresses included oxidative stress [sodium arsenite (30 µM) and hydrogen peroxide], ionizing radiation (γ-rays and neutrons), non-DNA damaging stresses [Tetradecanoylphorbol 13-acetate (TPA), heat shock, and osmotic shock], and other DNA-damaging stressors [adriamycin, camptothecin, cis-platin, methyl methanesulfonate (MMS), and UVB]. The resulting gene expression data were filtered for targets with good quality and intensity of hybridization across all experiments,\(^1\) and for significant change in expression in at least ten percent of the experiments. This yielded a set of 1451 genes that were used in subsequent analyses. Interestingly, when the information from this set of responsive genes was displayed in a multi-dimensional scaling (MDS) plot, a general grouping corresponding to the treatment classes above was apparent. Using a gene selection approach combining the results from maximum pairwise t-statistic, distance-based method, class-correlation and Wilcoxon/Kruskal-Wallis Statistic gene selection algorithms, we identified a set of 346 genes that gave the best discrimination between these four groups.

When the same analysis was repeated with the addition of new data from two independent treatments with 50 µM CdCl\(_2\), the gene expression response to CdCl\(_2\) was found to be very similar to that of sodium arsenite, the other heavy metal used in these experiments. MDS with the previously selected 346-gene signature clearly identified CdCl\(_2\) as an oxidative stress agent with a gene expression pattern very similar to that of arsenite.

Using the same gene selection algorithms, a signature of 251 genes was identified that discriminated between the response to arsenite and all the other treatments in the study. Although the CdCl\(_2\) data were not used to define this profile, a high degree of similarity in the responses to arsenite and CdCl\(_2\) was again evident. The relative responses of the genes in this signature to arsenite, CdCl\(_2\) and the other stresses is illustrated in the heatmap in Figure 2A. The overall similarity of the heavy metal responses is also indicated by the close grouping of these treatments as visualized by multi-dimensional scaling (Fig. 2B). This suggests a generally robust heavy metal-defining signature.

While most of the genes in the arsenite-defining signature show great similarity in their response to both arsenite and CdCl\(_2\), it also appears that some of these genes respond to arsenite to a much greater extent (Fig. 2A). Using the same gene selection techniques as above, we were able to identify 73 genes that discriminated between the arsenite and

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\(^1\) National Institutes of Health, National Cancer Institute, Division of Basic Science, Bethesda, MD.
CdCl₂ responses (Fig. 3). Genes responding to arsenite but not CdCl₂ and genes responding to CdCl₂ but not arsenite are both evident. Thus, while a robust general heavy metal signature is apparent, there is also a further layer of specific response evident.

CdCl₂ responses (Fig. 3). Genes responding to arsenite but not CdCl₂ and genes responding to CdCl₂ but not arsenite are both evident. Thus, while a robust general heavy metal signature is apparent, there is also a further layer of specific response evident.

It is possible to further explore these gene expression signatures in terms of the cellular roles and molecular functions of the proteins encoded by the responding genes. One approach to this involves comparison of the Gene Ontology (GO) annotations of the gene response signatures. The Expression Array Systematic Explorer (EASE) tool allows determination of statistically overrepresented Gene Ontology categories within a gene set compared with the frequency among genes on the array. Thus, this analysis identifies molecular functions and cellular processes represented in a set of genes more frequently than expected by chance alone, indicating possible key mechanisms involved in the process of interest. EASE analysis of the heavy metal-specific signature is summarized in Table I. Clearly, heatshock, protein folding, and metal ion-specific functions significantly dominated the heavy metal-specific signature. The molecular functions identified as significantly defining metal exposure are generally consistent with known modes of action and gene induction patterns. For instance, metallothioneins, heat shock proteins and genes involved in transcription have generally been found in microarray profiling studies to be major responders to both arsenite and cadmium stresses. It should be noted, however, that only regulation of individual genes from these categories was reported in these prior studies. There was no indication of the relative significance of functional classes in the overall response signature, and the

Fig. 2. Genes discriminating between arsenite and CdCl₂ and other (non-metal) stresses. (A) Heatmap showing expression levels of arsenite-discriminating genes. (B) Multidimensional scaling plot of the same metal-specific signature.

Fig. 3. Genes selected as discriminating between arsenite and cadmium exposures. Although there is overall great similarity in the response to heavy metals, some specificity is also apparent.
reporting gene responses did not distinguish metal stresses from any other mechanism of toxicity.

**References**


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**Stress-Specific p53-Dependent Responses**

*Sally A. Amundson, Khanh T. Do* and *Albert J. Fornace Jr.*

The tumor suppressor gene p53 is a major modulator of gene expression responses to many stresses, and its downstream effector genes regulate diverse cellular processes including apoptosis, DNA repair, cell cycle arrest and senescence. For instance, activation of p53 by ionizing radiation can trigger rapid apoptosis in cells of myeloid and lymphoid lineages, frequently correlating with p53-dependent gene induction, while cells derived from other tissues may be more prone to undergo cell cycle arrest. Crosstalk between stress-responsive signaling pathways may help determine the ultimate fate of exposed cells. For instance, MAPK pathways also regulate many p53-responsive apoptosis genes, and can contribute directly to regulation of p53. Hence, p53 signaling can be differentially regulated in different cellular contexts, or by the interaction of other signaling pathways activated by a specific stress agent. Such inter-pathway cross-talk may explain the responses of genes such as *GADD45A*, which requires wild-type p53 for response to ionizing radiation, but which is regulated by both p53-dependent and independent pathways following UV radiation or MMS exposure. Thus, p53-dependent responses can vary with exposure to different stress agents, depending on the activation of other signaling pathways.

As part of an effort to characterize molecular responses to diverse stressors, we have exposed two human cell lines (TK6, a p53 wild-type lymphoblastoid cell line, and NH32, its p53-null derivative) to a panel of 13 different chemical or physical agents. Microarray analysis was used to compare gene expression responses in these cell lines to oxidative stress (sodium arsenite, cadmium chloride and hydrogen peroxide), ionizing radiation (γ-rays and neutrons), non-DNA damaging stresses [Tetradecanoylphorbol 13-acetate (TPA), heat shock, and osmotic shock], and other DNA-damaging stressors [adriamycin, camptothecin, cis-platin, methyl methanesulfonate (MMS), and UVB]. The resulting gene expression data were filtered for targets with good quality and intensity of hybridization across all experiments, and for significant change in expression in at least ten percent of the experiments. This yielded a set of 1451 genes that were used in subsequent analyses. When the information from this set of responsive genes was displayed in a multi-dimensional scaling (MDS) plot without selection, no overall influence of cellular p53 status was apparent (Fig. 1). In contrast, there was an apparent general grouping corresponding to the treatment classes. From our previous work with ionizing radiation responses, in which the p53 signature is extremely prominent, it was surprising to find that the stress-specific signature completely dominated any p53-specific signature in these experiments.

Using a gene selection approach combining the results from maximum pairwise t-statistic, distance-based method, Wilcoxon/Kruskal-Wallis Statistic and class-correlation gene selection algorithms, we identified gene expression

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*1 National Institutes of Health, National Cancer Institute, Division of Basic Science, Bethesda, MD.*

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**Table I. Over-represented functions within metal-specific gene expression signature**

<table>
<thead>
<tr>
<th>Gene Ontology Category</th>
<th>*EASE Score</th>
<th>*Fisher Exact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat shock protein activity</td>
<td>0.000081</td>
<td>0.0000064</td>
</tr>
<tr>
<td>Transcription cofactor activity</td>
<td>0.00067</td>
<td>0.00019</td>
</tr>
<tr>
<td>Copper ion homeostasis</td>
<td>0.00072</td>
<td>0.000016</td>
</tr>
<tr>
<td>Transition metal ion homeostasis</td>
<td>0.0024</td>
<td>0.00011</td>
</tr>
<tr>
<td>Protein folding</td>
<td>0.0026</td>
<td>0.00055</td>
</tr>
<tr>
<td>Heavy metal sensitivity/resistance</td>
<td>0.0053</td>
<td>0.000079</td>
</tr>
<tr>
<td>Copper/cadmium binding</td>
<td>0.0057</td>
<td>0.000088</td>
</tr>
</tbody>
</table>

*Statistics calculated using the Expression Array Systematic Explorer.*

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**References**


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Using a gene selection approach combining the results from maximum pairwise t-statistic, distance-based method, Wilcoxon/Kruskal-Wallis Statistic and class-correlation gene selection algorithms, we identified gene expression

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*1 National Institutes of Health, National Cancer Institute, Division of Basic Science, Bethesda, MD.*
signatures that discriminated individual stresses or agent classes, including TPA, arsenite, and DNA-damaging agents. Most of these signatures comprised more than 100 genes. In contrast, using these same methods we identified only 13 genes that discriminated p53 status across the entire data set. When the resulting MDS plot was examined, however, it became evident that while the genotoxic treatments elicited a strong p53-dependent response, the non-genotoxic treatments remained clustered by treatment, and did not show the same p53-dependent pattern as the genotoxic treatments (Fig. 2).

The analysis of p53 effects was therefore done again using only data from the genotoxic treatments. This approach identified a 16-gene signature broadly discriminating p53 status across the genotoxic agents. This new signature included the 13 genes originally identified from the entire data set, with the addition of BTG1, BTG2 and ST14. The effect of p53 status on gene expression in response to exemplar genotoxic agents (γ-ray, MMS and UVB) is contrasted with the responses to non-genotoxic stressors in Figure 3.

Further analysis using only data from the non-genotoxic treatments could not identify any significantly p53-dependent genes among the responses to non-genotoxic agents. This suggests that the p53 pathway did not play a major role in gene expression responses in the absence of early DNA damage.

As p53 can be a major determinant of gene induction patterns following many types of stress, these findings suggest that a stress agent’s mechanism of action may be a more dominant determinant of gene expression patterns than cellular genotype. By extension, it may be that even inter-individual differences have less impact on agent-specific gene expression profiles. So, while p53 strongly regulates a subset of genes in response to specific stresses, the majority of stress-regulated genes appear to be p53-independent. This implies that the development of diagnostic gene expression signatures for specific toxic exposures may be feasible without undue confounding by individual genotype differences.

References

1. Zhan Q, Alamo I Jr, Yu K, Boise LH, O’Connor PM and Fornace AJ Jr. The apoptosis-associated gamma-ray re-


Some members of Dr. Hei’s lab focusing on the functions of the Betaig-h3 gene and low LET induced bystander effect (l-r): Genzi Shao, Joseph Gillispie, Sarah Huang, Rudranath Persaud and Yong-liang Zhao.

Dr. Yin’s lab focuses on functions of tumor suppressor genes (l-r): Wenhong Shen, Yuxin Yin and Alan Wong.
Histone H2AX Is Dispensable for Base Excision Repair Activity

Adayabalam S. Balajee, Rajamanickam Baskar and Charles R. Geard

H2AX, a variant form of histone H2A, is rapidly phosphorylated at serine 139 in response to DNA double strand break (DSB) inducing agents.\textsuperscript{[1–2]} Phosphorylated histone H2AX, designated as γ-H2AX, forms distinct nuclear foci at or near the DSB sites and is considered to be important for subsequent recruitment of additional DSB repair factors such as Nbs1 (Nijmegen breakage syndrome gene product) and 53BP1 (p53 binding protein 1).\textsuperscript{[3]} Although γ-H2AX is dispensable for the initial recognition of DSB,\textsuperscript{[4]} mouse embryonic stem cells and fibroblasts deficient in H2AX display enhanced chromosomal radiosensitivity.\textsuperscript{[5]} As ionizing radiation induces a wide spectrum of DNA lesions comprising DNA strand breaks and oxidized base lesions, it is difficult to determine whether the radiosensitivity of H2AX null cells stems from a deficiency either in DSB repair or a in base excision repair (BER) pathway or both. Identification of the repair deficiency will be of critical importance to understand the molecular pathway(s) with which H2AX functions as a dosage dependent suppressor of oncogenic chromosomal translocations and tumor formation.\textsuperscript{[6]} In this study, we have tested base excision repair activity in H2AX proficient and deficient mouse embryonic stem (ES) cells by a combination of \textit{in vivo} and \textit{in vitro} assays.

Mouse ES cells differing in the functional status of H2AX [wild type (+/+) , heterozygous (+/-) and null (-/-)] were generously provided by Drs. C.H. Bassing and F.W. Alt (Department of Genetics, The CBR Institute for Biomedical Research, The Children’s Hospital, Harvard Medical School, Boston, MA 02115, USA). The procedure followed for the generation of these cells lines as well as their growth characteristics have been described by Bassing et al.\textsuperscript{[5]} To determine the sensitivity of H2AX proficient and deficient cells to oxidative DNA damage, clonogenic survival experiments were performed after treatment with different concentrations of hydrogen peroxide. For survival experiments, 500 cells from wild type H2AX, heterozygous and null cell lines were seeded in gelatin coated 6cm dishes 18hr prior to treatment. Cells were treated with different concentrations of hydrogen peroxide (10 µM, 25 µM and 50 µM) for 30 min in complete medium at 37°C. After treatment, cells were washed twice in medium and the cells were grown for 10–12 days in complete medium. For comparison, we also did a survival experiment with different doses of gamma-rays. Although H2AX null cells were extremely sensitive to ionizing radiation as demonstrated earlier,\textsuperscript{[5]} no marked differences were observed among H2AX wild type, heterozygous and null cells in terms of sensitivity to hydrogen peroxide (Fig. 1).

We next determined cell cycle regulation in ES cells after oxidative DNA damage. ES cells were treated with different concentrations of hydrogen peroxide and the cells were processed for flow cytometry at 3hr, 6hr and 24hr after treatment. Cells were stained with propidium iodide and analyzed for cell cycle progression. Consistent with clonogenic survival data, the cell cycle profiles observed at 3hr (Fig. 2A), 6hr (Fig. 2B) and 24hr (Fig. 2C) after hydrogen peroxide treatment were grossly similar in all the three cell lines. Both H2AX proficient and deficient cells showed a concentration dependent G2/M phase arrest (~40%...
Fig. 2. Cell cycle analysis of H2AX proficient and deficient ES cells after treatment with hydrogen peroxide (A – 3hr; B – 6hr; C – 24hr after treatment) and gamma-rays (D – 6hr after IR).

and 55% of total cells) 24hr after treatment with 50 µM and 250 µM hydrogen peroxide (Fig. 2C). Further experiments are in progress using mitosis specific phosphorylated Histone H3 antibody to determine whether or not progression from G2 to M phase is altered in H2AX null cells. For comparison, ES cells treated with different doses of gamma-rays were also analyzed after 6 hr by flow cytometry. In accordance with previously published results, IR induced a dose dependent accumulation of G2/M phase cells in all the three cell lines with different H2AX status (Fig. 2D).

We next investigated the base excision repair capacity of H2AX wild type and null cells using an oligonucleotide based in vitro incision assay. Modified oligonucleotides as well as the unmodified complementary oligonucleotides were procured from Trevigen. Modified oligonucleotides (2 pmol) were 5'-end-labeled with gamma 32P-ATP using T4 polynucleotide kinase. After labeling, unincorporated nucleotides were removed by MicroSpin G-25 columns (Amersham) and the labeled oligonucleotides were mixed with a two-fold excess of unlabeled complementary oligonucleotides. The mixture was heated to 95°C in an annealing buffer and allowed to cool down to room temperature. An in vitro incision assay was carried out in 15 µl of reaction mixture containing 0.5 nmol oligonucleotide duplex, 25–50 µg of whole cell extract, 45 mM Hepes-KOH pH 7.8, 70 mM KCl, 1 mM dithiothreitol and 2 mM EDTA for 2 h at 37°C. The reaction was terminated by the addition of an equal volume of formamide dye (0.1% xylene cyanol, 0.1% bromophenol blue dissolved in 100% formamide) and incubated for 5 min at 90°C. The samples were run on a 20% polyacrylamide gel containing 7M urea, 89 mM Tris-borate, pH 8.0 and 2 mM EDTA. After electrophoresis, the gel was dried and exposed to x-ray films. The efficiency of incision was calculated by measuring the band intensity at the position of 10-mer. Reactions carried out in the absence of WCE served as negative controls.

In our study, we used four different types of modified oligos containing THF-AP site (tetrahydrofuran apurinic-apyrimidinic site), Uracil, 8-Oxo-dG (8-oxodeoxyguanine) and AP-site. Although the relative incision rates varied for different lesions, no major differences were observed in the in vitro BER incision capacity when H2AX wild type and null cells are compared (Fig. 3). Uracil, AP and THF-AP sites were efficiently incised (70%) by both H2AX wild type and null cells while only 20–25% incision activity was observed for 8-Oxo-dG. The in vitro incision assay clearly illustrates that H2AX null cells are quite competent in BER activity. We are in the process of determining the incision activity for other oxidative DNA lesions such as Hypoxanthine, Etheno-Adenosine and Etheno-Cytosine. Our results suggest that the observed radiosensitivity of H2AX
null cells is more likely due to misrepair or lack of repair of DSBs rather than a deficiency in BER.

References


Mouse ES Cells Devoid of Mrad9B are Sensitive to UV Radiation

Corinne Leloup, Aiping Zhu, Kevin M. Hopkins and Howard B. Lieberman

Human HRA9D and mouse Mrad9B were identified in our laboratory as paralogues of the cell cycle checkpoint control genes HRA9D and Mrad9, respectively.\(^1\) Mrad9 as well as HRA9D have a role in maintenance of genomic integrity and protection from DNA damage induced by radiations and certain chemicals. They also contribute to gamma-ray-induced G2 checkpoint delay. Furthermore Mrad9 regulates embryonic development.\(^2\)

The molecular mechanisms of their actions are starting to be unraveled. HRAD9 binds to HHUS1 and HRAD1\(^3\) and this complex associates with chromatin after DNA damage.\(^4\) HRAD9 activity at the G1/S checkpoint is dependent upon phosphorylation by ATM,\(^5\) and HRAD9 can activate the transcription of p21 in a p53 independent manner.\(^6\)

HRAD9B is nuclear and can also physically interact with checkpoint proteins HRAD1, HRAD9, HHUS1 and HHUS1B, like HRAD9. The mouse and human genes are expressed mainly in the testis but expression is reduced in testicular tumors.\(^1\)

In order to learn more about the function of Mrad9B, mouse embryonic stem cells containing the first two exons of the gene replaced by the neomycin resistance gene were constructed and characterized.

Generation of knock-out ES cell lines

ES cells were transfected with a targeting construct and selected for resistance to neomycin (150 µg/ml G418). Resistant clones have been isolated, and 4 out of 130 clones examined were heterozygous for the targeted locus.

![Fig. 1. Southern blot analysis of the Mrad9B gene in mouse ES cells. WT: wild type; Het: heterozygous; Homo: homozygous.](image)

Southern blot analysis of the region downstream of the target site demonstrated the Mrad9B heterozygosity of the clones (Fig. 1). These results were confirmed by PCR analysis (data not shown).

The heterozygous clones were challenged with 800 µg/ml G418 and analyzed by Southern blotting. The neomycin resistance gene present will mediate resistance to high levels of the drug if two copies of the gene are present. One drug-resistant clone out of 35 displayed the Southern blot pattern predicted for the homozygous replacement of the first and second Mrad9B exons by the neomycin gene (Fig. 1). Northern blot analyses indicated that Mrad9B expression was abolished in these ES cells (data not shown).

**Mrad9B-/- cells are sensitive to UV radiation**

We found that Mrad9B+/- ES cells are slightly more sensitive to UV radiation compared to the wild type control. Homozygous Mrad9B mutant ES cells are even more sensitive (Fig. 2).

These experiments thus indicate that Mrad9B is important for promoting resistance to damage caused by UV. Mrad9B-/- ES cells are also more sensitive to γ-rays, relative to the wild-type control (data not shown).

Experiments are underway to define the mechanisms involved in Mrad9B-mediated radioresistance, and whether the encoded protein also influences the cellular response to other DNA damaging agents.

**References**


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**Effect of Haploinsufficiency on Oncogenic Transformation and Survival in Mouse Embryo Fibroblast Cells**

*Stephen A. Mitchell, Lubomir Smilenov, Howard B. Lieberman and Eric J. Hall*

**Introduction**

Several recent studies have suggested that heterozygosity leading to haploinsufficiency for proteins involved in DNA repair pathways plays a critical role in genomic instability and carcinogenesis.[1–5] The present study was carried out to investigate the effect of varying genetic background clonogenic survival and oncogenic transformation. Mouse embryo fibroblasts (MEF) from animals haploinsufficient for one or more genes involved in DNA repair and/or checkpoint controls,[6] namely Atm, BRCA1 and Mrad9 were studied. ATM is a sensor/transducer protein which is activated immediately after DNA DSB induction and it phosphorylates a number of proteins involved in repair and damage signaling pathways following damage (reviewed in Shiloh, 2003).[7] BRCA1 is phosphorylated following irradiation and is part of a complex containing ATM and
irradiation led to a modest increase in the transformation of MEF after mice, laboratory have shown that heterozygosity for at least transduction pathways.

regulating multiple radioresponses as part of signal

fission yeast formation. subsequently found as orthologues in humans

complex where it is thought to function as a scaffold for repair proteins. The RAD9 gene, first identified in the fission yeast Schizosaccharomyces pombe, then subsequently found as orthologues in humans and in mice, Mrad9 the mouse version, is an important element regulating multiple radioresponses as part of signal transduction pathways. Previous results from this laboratory have shown that heterozygosity for at least Atm led to a modest increase in the transformation of MEF after irradiation and was also a factor in cataract formation.

Materials and Methods

To assess clonogenic survival and transformation frequency, exponentially-growing MEF cells were exposed to 250 kVp x-rays at 15 mA with 0.5 mm copper and 1 mm aluminum external filters. Control cells were sham irradiated. The absorbed dose rate was calculated to be 1.2 Gy min⁻¹. Clonogenic survival was assessed up to a dose of 6 Gy and transformation at 0, 0.1, 1 and 2 Gy. Following irradiation, approximately 100 viable cells were plated into 100 mm culture dishes containing a heavily irradiated (30 Gy x-rays) feeder layer. The total number of cells (viable plus feeder) was 70,000 per dish. Dishes were incubated for 2 weeks in DMEM supplemented with 10% FBS without medium change and the resulting colonies were stained with Giemsa to determine both the plating efficiencies and surviving fractions of the control and irradiated cells. Transformed clones appeared as dense colonies containing stellate-shaped cells. Data from a minimum of three

Clonogenic survival, number of viable cells exposed in transformation studies, number of transformed clones produced and transformation frequencies for mouse embryo fibroblast cells irradiated with 250 kVp x-rays.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Dose (Gy)</th>
<th>Clonogenic surviving fraction (plating efficiency)</th>
<th>No. of viable* cells exposed</th>
<th>No. of transformants produced</th>
<th>Frequency of transformants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atm hz</td>
<td>0 Gy</td>
<td>(0.02)</td>
<td>27120</td>
<td>3</td>
<td>0.01</td>
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<tr>
<td></td>
<td>0.1 Gy</td>
<td>0.97±0.02</td>
<td>25984</td>
<td>4</td>
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<tr>
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<tr>
<td></td>
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<td>0.51±0.04</td>
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<td>0.21</td>
</tr>
<tr>
<td>Atm wt/Mrd9 hz</td>
<td>0 Gy</td>
<td>(0.01)</td>
<td>22100</td>
<td>3</td>
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</tr>
<tr>
<td></td>
<td>0.1 Gy</td>
<td>0.98±0.01</td>
<td>26617</td>
<td>4</td>
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<tr>
<td></td>
<td>1 Gy</td>
<td>0.72±0.01</td>
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</tr>
<tr>
<td>Atm hz/Mrd9 hz</td>
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<td>(0.02)</td>
<td>16630</td>
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<tr>
<td></td>
<td>0.1 Gy</td>
<td>0.96±0.03</td>
<td>24716</td>
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<tr>
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<td>Atm wt/Mrd9 wt</td>
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<tr>
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<td>0.30±0.04</td>
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<td>32</td>
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</table>

* Estimated, accounting for plating efficiency and clonogenic survival.

independent experiments were pooled and results were presented as a mean and either standard error for survival or 95% confidence limits for transformation.

Results and Discussion

No significant differences between genotypes were observed for clonogenic survival using a multiple regression analysis (Fig. 1). However, the results in Table I and Figure 2 indicate that a higher transformation frequency was observed for the single and double heterozygous cells compared with wild-type cells (e.g., Atm hz/mRd9 hz: P<0.0001). Cells heterozygous for both ATM and Rad9 were the most sensitive to radiation oncogenesis, having a transformation frequency almost three times that of the corresponding wild type cells. In addition to an increased sensitivity to radiation oncogenesis, cells with this genotype exhibit altered double-strand break repair dynamics and less apoptosis when compared with wild type cells or those haploinsufficient for only one of these proteins. These results suggest that following a stress insult, the cellular response is dependent on the expression levels of
closely interacting proteins which function in related pathways. As observed for mice heterozygous for both ATM and Rad9, the additive effect of a second haploinsufficiency may significantly contribute to the destabilization of a cell signaling network.

References

Role of EGFR Signaling in the Regulation of Arsenite-Induced Apoptosis of Human Melanoma and Prostate Carcinoma Cell Lines

Vladimir N. Ivanov and Tom K. Hei

Many therapeutic approaches, including γ-irradiation and chemotherapeutic drug treatment, have been proposed to reactivate apoptosis in cancer cells. Human malignant melanomas, an often deadly form of skin cancer due to the lack of effective treatment options, possess numerous genetic and epigenetic mechanisms that suppress apoptosis, which allows tumor survival after treatment.[1–3] In our recent study, we elucidated pro-apoptotic activities induced by arsenite in human melanomas and demonstrated that arsenite-mediated NF-κB inhibition and simultaneous endogenous expression of death receptor ligand TNFα[5] sensitize melanoma cells to undergo TNFα-mediated apoptosis.[5] The next task of our study was to develop an effective combined treatment of cancer cells using arsenite and specific inhibitors of cell survival pathways.

Epidermal growth factor receptor signaling has become an important target in anticancer drug development due to its ability to control tumor cell proliferation and to suppress apoptosis.[6] Extensive efforts to develop EGFR inhibitors for anticancer therapy[7–10] finally led to approval of one of the small reversible inhibitors, gefitinib (Iressa, AstraZeneca), for non-small cell lung cancer treatment and of an inhibitory anti-EGFR monoclonal antibody, IMC-C225 (Eributx, ImClone Systems), for metastatic colorectal cancer treatment. Activation of the EGFR pathway in cancer cells is based on several distinct mechanisms, such as amplification and/or overexpression of the EGFR gene, increased production of ligands, EGF or TGFα, decreased receptor turnover, and the presence of altered forms of receptors due to specific activating mutations or profound gene rearrangement. Effects of EGFR signaling on cell proliferation and survival are mediated by the MAPK, PI3K-AKT and STAT pathways.[8,11–12,6]

Epidermal growth factor receptor (EGFR) is expressed, albeit at low or intermediate levels in human melanomas at the different stages of tumor progression. Coexpression of EGFR with its ligand TGFα indicates their role in paracrine and autocrine growth regulation of melanomas. As it was previously observed for several types of cancer, specific inhibitors of EGFR-mediated signaling may reduce anti-apoptotic properties of cancer cells and sensitize them to cytotoxic drugs. We recently reported that arsenite, particularly in combination with inhibitors of the PI3K-AKT and MEK-ERK pathways, induces high levels of apoptosis in different melanomas.[5] Since EGFR signaling operates via activation of the PI3K-AKT and MEK-ERK pathways, we suggested that the combination of arsenite and EGFR inhibitors might also effectively induce apoptosis in melanoma. Here we demonstrate that a moderate concentration of arsenite (5–10 μM) indeed up-regulates apoptosis induced by EGFR inhibitors in EGFR-positive melanomas. In contrast, induction of apoptosis in melanomas with negligible surface expression of EGFR or with defective EGFR signaling requires direct suppression of the PI3K-AKT and MAPK pathways by specific pharmacological inhibitors in the presence of arsenite. Under these conditions, metastatic melanoma cell lines undergo TRAIL- and TNFα-mediated apoptosis. Taken together, these data provide additional approaches sensitizing melanomas to cytotoxic effects of specific inhibitors of survival pathways.

There is an obvious mechanistic analogy in the general arrangement of pro- and anti-apoptotic signaling between melanomas and prostate adenocarcinomas. In this respect, androgen-dependent LNCaP cells with low basal NF-κB activity and high sensitivity to TNFα- or arsenite-induced apoptosis are similar to early WM793 melanoma cells. On the other hand, highly aggressive androgen-independent DU145 prostate cancer cells with a high basal NF-κB activity and resistance to both TNFα- or arsenite-induced apoptosis are quite similar to metastatic melanoma L1205. For both cell systems, down-regulation of the basal NF-κB activities by different agents may induce TNFα and TRAIL-mediated apoptosis.[13] Surprisingly, in spite of the high surface expression of EGFR in PC3 and DU145 prostate cancer cells, inhibitors of EGFR (alone or in concert with arsenite) induced only modest levels of apoptosis in these cells, while LNCaP cells were highly sensitive to arsenite alone or in concert with EGFR inhibitor (Fig. 1). That was opposite to EGFR-positive melanomas, which are highly responsive to the combined treatment of EGFR inhibitors and arsenite, as was shown in this study. This discrepancy may reflect potential mutations and alterations of the EGFR gene in prostate cancer cell lines as have been recently reported in non-small lung cancer cells.[14–15]

References

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**Fig. 1.** (A) Levels of EGFR in prostate cancer cells. (B) Apoptosis levels 24h after treatment with indicated stimuli.
Quantification of CD59<sup>−</sup> Mutants of Human-Hamster Hybrid (A<sub>L</sub>) Cells by Using Flow Cytometry

Hongning Zhou, An Xu, Joseph A. Gillispie, Charles A. Waldren<sup>1</sup> and Tom K. Hei

Determining the carcinogenic effect of chemicals to humans is an important issue related to cancer prevention. Unfortunately, epidemiology and animal studies are unavailable for most individual chemicals. Mutagenesis tests can help in predicting carcinogenicity, not only because of the correlation between the mutation assay and potential carcinogenesis, but also the relatively short period to compare with the traditional carcinogenic experiments. However, many short-term assays are inaccurate and others are laborious and expensive. Among these mutagenic assays, A<sub>L</sub> cells developed by Puck and co-workers provided a sensitive system for mutation analysis.

The human-hamster hybrid (A<sub>L</sub>) cell system is a well-established in vitro model for detecting mutants that induce mostly large, multilocus deletions, as well as small deletions, even point mutation. The A<sub>L</sub> cells contain a standard set of CHO chromosomes and a single human chromosome 11, which expresses several cell surface proteins including CD59 encoded by the CD59 gene at 11p13.5. CD59 is a widely distributed, glycosy/lphosphatidylinositol (GPI)-anchored cell surface protein, which acts as an inhibitor of complement. Because only a small part of 11p1 is required for the viability of A<sub>L</sub> cells, mutations in the human chromosome 11 ranging in size up to 140 Mbp of DNA can be detected. After exposure to mutagens, using rabbit serum-complement plus anti CD59 antibody, mutants lacking CD59 antigen can form colonies, while wild type cells are killed. However, it has been suggested that even mutated cells can maintain small amounts of wild type CD59 protein for several generations, which results in less efficient mutation detection. Furthermore, the frequency of spontaneous mutants was increased along with the period incubation. Usually, it takes 3–4 weeks to complete the whole analysis.

A modified mutation assay by flow cytometry has been developed to determine the yield of CD59 mutants. After being incubated with phycoerythrin-conjugated mouse monoclonal anti-CD59 antibody and rinsed, the fluorescence of the cells was detected by flow cytometry (FACSCalibur<sup>TM</sup>, Becton Dickinson) to quantify CD59 mutant frequency. The flow cytometry mutation assay could easily avoid the toxicity of complement. Moreover, it can shorten the period of experiments by about 1 week (colony formation period), and therefore reduce the labor and costs.

To test the separation efficiency of CD59 negative and positive cells by flow cytometry, stained wild type and mutant CD59 A<sub>L</sub> cells were prepared, as well as the unstained wild type and CD59 mutant A<sub>L</sub> cells. We found unstained wild type and CD59 mutant A<sub>L</sub> cells demonstrated the similar fluorescence as the stained CD59<sup>−</sup> mutant A<sub>L</sub> cells, all were considered as negative when the gate was set. For convenience, unstained A<sub>L</sub> cells were used as the negative control in the next experiments. However, the stained wild type A<sub>L</sub> cells showed very strong fluorescence. The background of the negative (mutant) cells was generally less than 0.15%. An example experiment is shown in Figure 1A, with the setting gate, 99.96% of the unstained A<sub>L</sub> cells were measured as CD59 negative (fluorescence below the line), while only 0.12% of the stained wild type A<sub>L</sub> cells located in this region, which means the background CD59 mutation in this experiment is 0.12% or 120 per 10<sup>5</sup> survivors. Also, it was shown the mean intensity of stained wild type A<sub>L</sub> cells is more than 500 times higher than that of unstained A<sub>L</sub> cells in Figure 1B. This test convinced us of the potential of flow cytometry to detect the CD59 mutants in the A<sub>L</sub> cell system.

To determine the accuracy of CD59 detection by flow cytometry, mutant A<sub>L</sub> cells were mixed with wild-type cells in known proportions (0.1–10% mutant cells) and these

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<sup>1</sup> Radiation Effects Research Foundation, Hiroshima, Japan.

Fig. 1. Representative data from the flow cytometry mutation assay. The gate set for this experiment showed that unstained A<sub>L</sub> cells show 99.96% of the cells that were CD59 negative (A), while stained wild type A<sub>L</sub> cells showed 99.88% of the cells that were CD59 positive (B).
samples were stained and passed through the flow cytometry. The results showed the high accuracy of CD59 detection with flow cytometry (data not shown).

Based on our previous experiments, gamma-ray irradiation could induce dose dependent mutagenic effects in A549 cells with a 7 to 10 day expression period. In the present study, gamma-rays caused dose dependent cell killing as reported previous (data not shown). After a 7-day expression period, cells are prepared for mutation assays using both traditional and flow cytometry techniques. As shown in Figure 2, both methods demonstrated dose dependent mutagenesis, with the increase of irradiation dose, the mutant yields were increased. However, the flow cytometry mutation assay showed higher induced mutation frequency than that of the traditional complement/antibody assay. These data suggested that the flow cytometry mutation assay might be more sensitive than the traditional method to assay CD59 mutations.

To further determine the accuracy of the flow cytometry method in detecting CD59 mutants, cells were treated with a well known mutagen, MNU. After a 7-day expression period, cells were prepared as described before. Similar results were found for MNU induced mutagenesis as for radiation. In addition to the dose response mutagenesis, the flow cytometry method also showed a higher induced mutant yields compared with the traditional assay. These results further confirmed that flow cytometry analysis had more sensitivity compared with the traditional assay.

Overall, our present study indicated that the flow cytometry assay for CD59 mutation in A549 cells is a reliable method, but more sensitive and efficient than the traditional assay. The spectrum of the mutants will also be assessed in the future.

**Establishment and Characterization of Mitochondrial DNA Deficient (ρ<sup>0</sup>) A<sub>549</sub> Cells**

Su X. Liu and Tom K. Hei

To ascertain the role of mitochondria in genotoxicity induced by radiation and chemical carcinogens, a mitochondrial DNA deficient (ρ<sup>0</sup>) cell line from A549 cells was generated using the chemotherapeutic drug ditercalinium. Exponentially growing A549 cells were cultured in F12 medium containing 1.5 µg/ml of ditercalinium for 3 months in the presence of uridine (50 µg/ml). Although the majority of A549 cells died during the course of treatment, clonal isolates began to form 2–3 weeks after drug treatment. Colonies, formed from drug resistant cells, were isolated and be passaged in drug containing medium for 2 additional months. Several ρ<sup>0</sup> cell lines treated with ditercalinium for more than 5 months were established. The clonal isolates were fully characterized to be ρ<sup>0</sup> (mtDNA deficient) by several assays including i) PCR amplification using primers for mtDNA; ii) JC-1 stain for mitochondrial membrane potential.

To examine mtDNA in the ρ<sup>0</sup> cells generated with ditercalinium, total DNAs from ρ<sup>0</sup> cells were isolated, and primers were synthesized using the nucleotide sequence of the peptidyl transferase encoding region of the 16S rRNA
Arsenite is a known human carcinogen and induces a variety of human cancers of the lung, skin, bladder, kidney and liver. The mechanisms of cancer induction by arsenite remains poorly understood.

For studying the stepwise neoplastic transformation of human lung cancer induced by arsenite, the human small airway epithelial cells (SAEC) purchased from Clonetics (Wilkesville, MD) were transfected with human telomerase reverse transcriptase (hTERT), and immortalized.[1] The hTERT-expressing cells (SAEC-hTERT) at passage 10 after G418 selection were treated with a 2 µg/ml of arsenite for 8 weeks (SAEC-hTERT-Ars), and then cultured continuously in parallel with non-treated SAEC-hTERT cells for more than 150 population doublings (PDs). The SAEC-hTERT-Ars cells exhibited transformed phenotypes after 150 PDs; they had a higher proliferation rate and saturation density than control SAEC-hTERT cells. When the SAEC-hTERT-Ars cells were plated in soft agar, colonies were formed; in contrast, there was no colony growth in soft agar by the control SAEC-hTERT cells. Seven agar colonies were isolated and lines were established from hTERT-SAEC-Ars cells. The karyotype in 2 of 7 agar cell lines together with control hTERT-SAEC cells at their early passage 30 and later passage 50 were analyzed with Giemsa stain.

Results showed that the karyotype of the control hTERT cells either at early passage or at later passage were near diploid. More than 80% of the cells had 46 chromosomes and less than 10% of the cells had 47 chromosomes (Fig. 1). However, the karyotype of the cells able to grow in the agar (SAEC-hTERT-Ars cells), only about 10% of the population with 46 chromosomes; 70–80% of the cells had 47

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chromosomes and 10–20% of the cells had 48 chromosomes (Fig. 2). The results of this karyotype analysis suggest that an increased copy of chromosomes in the cells of agar clones may be related to the transformed phenotype induced by arsenite. Tumorigenicity in nude mice will be analyzed for the 7 agar cell lines.

References


A Comparison of Genotoxicity between Arsenic and Dimethylarsenic Acid (DMA\textsuperscript{V}) in A\textsubscript{L} Cells

Su X. Liu and Tom K. Hei

In mammals, inorganic arsenic can be biomethylated to monomethylarsenic acid (MMA\textsuperscript{III}), monomethylarsonous acid (MMA\textsuperscript{II}), dimethylarsinic acid (DMA\textsuperscript{V}), dimethylarsonous acid (DMA\textsuperscript{III}), and trimethylarsine oxide. Levels of many of these organo-metallic forms of arsenic have been found in the urine of humans exposed to arsenic in their drinking water. Although biomethylation of inorganic arsenic was thought initially to be a detoxification process, this notion has been questioned because the methylated trivalent arsenic metabolites MMA\textsuperscript{III} and DMA\textsuperscript{III} have been shown to be toxic to mammalian liver, skin, urinary bladder and lung cells in culture. However, the toxicity of DMA\textsuperscript{V} is not clear.

In this study, the A\textsubscript{L} cell mutagenic assay was used to compare the surviving fraction, CD59\textsuperscript{-} mutations and mutant spectra induced by arsenic or DMA\textsuperscript{V}. Figure 1 shows the surviving fraction of A\textsubscript{L} cells treated with graded doses of arsenite or DMA\textsuperscript{V} for 24 h. The results showed that the toxicity of DMA\textsuperscript{V} was much lower than arsenite. The surviving fractions of A\textsubscript{L} cells treated with the arsensical compounds were about 62% by 7.5 \(\mu\text{M}\) of arsenite and 28% by 15 \(\mu\text{M}\) of arsenite. In contrast, 73% and 52% of cells were clonogenic after treatment with 0.5 and 1.0 mM of DMA\textsuperscript{V}, respectively. Comparing the 1 day or 5 days treatment with DMA\textsuperscript{V}, the toxicity was time dependent and a decrease in surviving fraction was observed in the 5 days treatment (Fig. 2).

Figure 3 shows CD59\textsuperscript{-} mutation induction in A\textsubscript{L} cells by graded doses of arsenite or DMA\textsuperscript{V}. The results showed that arsenite induced a much higher CD59\textsuperscript{-} mutation than DMA\textsuperscript{V}. The mutation frequency per \(10^5\) survivors was about 80 at 7.5 \(\mu\text{M}\) and 175 at 15 \(\mu\text{M}\) in cells treated with arsenite. In contrast, the mutant fraction was 60 at 500 \(\mu\text{M}\) and 120 at 540 \(\mu\text{M}\) of DMA\textsuperscript{V}.

Fig. 1. Surviving fraction treated with graded doses of arsenite or DMA\textsuperscript{V}.
1000 µM upon exposure to DMA\textsuperscript{V}. The mutation induction by DMA\textsuperscript{V} was also dependent on treatment period (as shown in Figure 4 by comparing 1 day and 5 days treatment results). The spectrum of CD59\textsuperscript{V} mutations induced by arsenite or DMA was analyzed by multiplex PCR. Although at doses of 7.5 µM of arsenite and 1000 µM of DMA\textsuperscript{V}, multiple marker loss was induced in the mutant cells compared with control, there was no obvious difference between the two arsenic-like compounds (Fig. 5).

Our findings suggest that DMA\textsuperscript{V} is significantly less toxic and mutagenic than inorganic arsenite.
Dr. Kevin Prise, of the Gray Cancer Institute, Middlesex, U.K., and Dr Hei.

Dr. Nicholas Geacintov and Dr. Suse Broyde, both of New York University, Dr. Victor Fung of the National Institutes of Health, Bethesda, MD, and Dr. Hei.

Dr. Hei, Dr. Takehiko Nohmi of National Institute of Health Sciences, Tokyo, Japan, and Dr. Lubomir Smilenov.

Dr. Hei and Dr. Zigang Dong of the Hormel Institute, Austin, Minnesota.

Dr. Hei and Dr. Gloria Calaf, an Adjunct Associate Research Scientist at the Center, who spends half the year as a professor at the University of Tarapaca, Chile.

Dr. Hei with three high-school students participating in a summer science program.
Cytogenetic Analysis of Lymphocytes from Mayak Workers


We have previously discovered a biomarker of past exposure to high-LET radiation in chromosomes of individuals working at the Mayak Production Association, Ozyorsk, Russia.[1-2] This “biomarker” takes the form of an increase in intra-chromosomal aberrations in lymphocytes of those exposed to Pu compared with those exposed to gamma radiation. mBAND analysis was used to examine aberrations in chromosome 1, 2 and 5. The response appears to be dose dependent in chromosome 5. This pilot study investigated whether a biomarker was present in 31 individuals and we have extended our study to include 300 individuals who worked at the Mayak facility. It is hoped with the inclusion of more individuals a quantitative biodosimeter can be developed. This could then be used to increase the accuracy of epidemiological estimates of high-LET radiation exposed individuals such as nuclear workers, airline flight crew, astronauts and patients undergoing neutron radiotherapy.

At present the lymphocytes from 50 individuals have been examined for the presence of intra-chromosomal aberrations in chromosomes 2 and 5. As samples from individuals are scored blind, the results will not be known until the chromosomes of all 300 individuals have been analyzed. Figure 1 shows an example of an intra-chromosomal aberration found in one of the workers.

References


Fig. 1. Intra-chromosomal aberration detected in chromosome 5 of a PBL of a Mayak worker. Left chromosome is normal and the right shows the aberration. Grey arrows denote the centromeres. Yellow arrows indicate the region of the chromosome which was inverted, as indicated by the reversal in the sequence of band colors. This is an example of an inter-arm intra-chromosomal aberration (pericentric inversion). The tip of the right chromosome is also missing (purple band). This indicates that there is also a terminal deletion.

1 Southern Urals Biophysics Institute, Ozyorsk, Russia.
Genetic Susceptibility to Cataract Induction by High Energy Heavy Ions

Eric J. Hall, David J. Brenner, Basil V. Worgul and Lubomir Smilenov

The only degenerative effect experienced so far by astronauts as a legacy of their radiation exposure during space flight is the early onset of ocular cataracts.

In the context of space radiation, it is important to know whether the human population includes genetically predisposed radiosensitive subsets. One possibility is that haploinsufficiency of ATM confers radiosensitivity, and this defect involves 1–3% of the population.

In the context of Space Radiation Risk Assessment, the existence of an unidentified radiosensitive sub-population would have two consequences. First, it might be considered unethical to put radiosensitive individuals into situations where they might receive large doses of radiation because of the possible severe clinical response. Second, the existence of a radiosensitive sub-population in an epidemiological study would tend to distort the shape of the dose-response relationship, thereby rendering a linear extrapolation from high to low doses invalid.

Since human studies are notoriously difficult, so we turned our attention to knock-out mice, where the Atm gene is disrupted by inserting a neo cassette, with the consequence that there was no presence of full-length or truncated protein in the knock-out animals. One eye of wild-type and Atm heterozygous and knock-out mice was exposed to graded doses of high energy $^{59}$Fe ions at the Brookhaven Alternating Gradient Synchrotron. Eyes were examined over the next 18 months and cataract grades scored.

Data for cataract prevalence after exposure to a dose of 0.325 Gy of $^{59}$Fe ions, which corresponds to approximately one particle traversal per cell, are shown in Figure 1, which indicates the prevalence of low grade cataracts, and that cataracts appear earlier in Atm heterozygotes than in wild-type animals. Figure 2 shows the corresponding prevalence data for high grade vision-impairing cataracts. The dose used, 0.325 Gy, results in vision impairing cataracts in the majority of the Atm heterozygotes, but in essentially none of the wild-type animals.

These data on radiation-induced cataracts in mice of different genetic backgrounds assume additional significance because of the observation of an increased risk of cataracts in astronauts with higher lens doses (>8 mSv) of space radiation relative to astronauts with lower lens doses.[2]

Of even more interest is the fact that 35 of 39 cases of cataracts after space flight occurred in astronauts who participated in lunar missions or high inclination shuttle flights, possibly because of the higher flux of high energy heavy ions in these situations. The vast majority of the recorded cataracts involved opacities of the cortical and/or posterior subcapsular variety, which is highly and definably characteristic of radiation as the causative agent. Overall, 3 of the 295 astronauts followed developed vision-impairing cataracts that required surgery relatively early in life, even though the accumulated doses were quite low. It is interesting to speculate that this may indicate a genetically predisposed sensitivity in these individuals.

Fig. 1. Data for cataract prevalence after exposure to a dose of 0.325 Gy of $^{59}$Fe ions, which corresponds to approximately one particle traversal per cell, showing the prevalence of low grade cataracts, and that cataracts appear earlier in Atm heterozygotes than in wild-type animals.

Fig. 2. The corresponding prevalence data for high grade vision-impairing cataracts. The dose used, 0.325 Gy, results in vision impairing cataracts in the majority of the Atm heterozygotes, but in essentially none of the wild-type animals.
Late rectal bleeding is well established as a key dose-limiting endpoint in prostate radiotherapy, and is an important consideration in cervical cancer radiotherapy. Consequently, much effort has been devoted to establishing dose-response relations for rectal bleeding. Clinically, however, little has been directly established about the response of this endpoint to changes in fractionation (as quantified, for example, by the $\alpha/\beta$ ratio), though there have been increasing suggestions in the literature that at least part of the late rectal response is a “consequential” late effect, directly correlated with early rectal damage. If this is the case, one might expect that the response of this endpoint to changes in fractionation might be intermediate between a classic late effect (typical $\alpha/\beta$ value: 1 to 3 Gy) and a classic early response (typical $\alpha/\beta$ value: 8 to 10 Gy); and indeed the $\alpha/\beta$ ratios estimated for late rectal damage in rodents do seem to be in this intermediate range.

So new clinical data on the response of late rectal damage to changes in fractionation are of interest both from a mechanistic standpoint, and also because there has been much recent interest in hypofractionated prostate radiotherapy. Akimoto et al. report on late rectal bleeding after hypofractionated radiotherapy of the prostate, in which they delivered 69 Gy in 3 Gy fractions, using 3-D conformal radiotherapy (3D-CRT) without rectal blocking or explicit dose-volume histogram (DVH) based criteria. Akimoto et al. report a late RTOG grade 2 complication rate of 25% (mean follow up: 31 months), virtually identical to that reported by the M.D. Anderson Cancer Center using a 3D-CRT dose of 78 Gy delivered in conventional 2 Gy fractions. Akimoto et al. also report, as have others, that diabetes is a highly significant predictor of late rectal sequelae.

As well as being of interest in their own right, these new hypofractionation data allow, for the first time, a quantitative estimate of the $\alpha/\beta$ ratio for late rectal bleeding, quantifying how rectal bleeding risks would be expected to change with changes in fractionation. This is possible because there is already a considerable amount of data in the literature on late rectal bleeding after conventionally fractionated (1.8 or 2 Gy fractions) radiotherapy delivered with a similar technique to that of Akimoto et al. (i.e., 3D-CRT without rectal blocking or use of explicit DVH criteria). Because of the uniformity in fraction size among these earlier data, it has not been possible to make a quantitative estimate of the $\alpha/\beta$ ratio (i.e., the sensitivity to changes in fraction size). With the new 3 Gy fraction data, however, it is now possible to globally analyze the 1.8 Gy, 2 Gy and 3 Gy per fraction data using the standard linear-quadratic model to generate an estimate of the $\alpha/\beta$ ratio for late rectal bleeding.

There are many reports in the literature of late GU sequelae risks after 3D-CRT with conventional fractionation; we chose to analyze the three largest series in the US, from the MD Anderson Cancer Center (MDACC), from Memorial Sloan-Kettering Cancer Center (MSKCC), and from RTOG protocol 9406, each of which used a similar approach to treatment planning to that of Akimoto et al. The highest dose points in the MSKCC and RTOG studies (81 and 79 Gy respectively) were excluded as rectal blocks or DVH-based treatment planning were utilized. We also excluded the corresponding Fox Chase Cancer Center series as almost all of their 3D-CRT patients were treated using rectal blocks. We excluded all French, British, and Australian studies, as the diabetes prevalence in those countries is dramatically different from those in the US and Japan (estimated year 2000 prevalences above age 20 in the USA, Japan, UK, France, and Australia, are, respectively, 6.9%, 7.6%, 2.1%, 2.1%, and 2.7%).

Thus the new Japanese data, together with the MDACC, MSKCC, and RTOG data were fitted to the standard linear-quadratic formalism for normal-tissue complications in which the probability of RTOG grade $\geq$ 2 late rectal toxicity, $P_{\text{RTOG2}}$, is written in terms of the number, $K$, of tissue rescuing units, and their survival, $S$, after a dose $D$ delivered in $N$ fractions:

$$P_{\text{RTOG2}} = \exp (-K S), \quad S = \exp (-\alpha D - \beta D^2 / N).$$

Data fitting used standard iteratively-reweighted least squares, with parameter confidence limits estimated using synthetic data simulation. The estimated value of $\alpha/\beta$ value for RTOG grade $\geq$ 2 late rectal toxicity is $5.4 \pm 1.5$ Gy. Figure 1 shows the data and the model fit, in which all the doses have been “converted” to equivalent 2 Gy fractionated doses, using the 5.4 Gy $\alpha/\beta$ value.
This α/β estimate—which can also be roughly estimated with a “back-of-the-envelope” calculation based on the equivalent rectal toxicity of 69 Gy in 3 Gy fractions— is indeed intermediate between typical values for early and late responding tissues, giving credence to the notion that at least some late rectal damage is a direct consequence of early-responding damage. The 5.4 Gy α/β value is also consistent with most estimates for late rectal damage in rodents.

As well as being of interest from a mechanistic standpoint, the α/β estimate allows us to predict, with more confidence, the risks of late GU complications for alternate fractionation schemes. For example, the estimated 5.4 ±1.5 Gy α/β value for late rectal damage may well be higher than that for most prostate cancers (where α/β estimates have generally been in the range from 1 to 3.5 Gy). This suggests that hypofractionation, at the appropriate dose, as well as being logistically convenient, might actually improve the therapeutic outcome of prostate cancer radiotherapy.

References


Mass Screening with CT Colonography: Should the Radiation Exposure be of Concern?

David J. Brenner

Mass Screening with CT Colonography (CTC), sometimes referred to as “virtual colonoscopy,” is attracting a great deal of attention as a potential option for mass screening for colon cancer. In its most common current usage, after bowel preparation, the colon is inflated and the colon is CT scanned. The resulting data can then be analyzed for polyps based on two-dimensional images, or via a three-dimensional endoluminal view. In general, CTC is an excellent application of CT because of the excellent contrast for polyps based on two-dimensional images, or via a three-dimensional endoluminal view.

A major advantage of CTC is its potential to increase compliance with current colonoscopy screening guidelines, particularly if non-cathartic bowel preparation techniques become standard for CTC. Current compliance is clearly suboptimal – at most, about one third of adults over 50 in the US have had an endoscopic examination within the past ten years.

From a technological perspective, CTC is not quite ready for use in mass-screening programs, for two main reasons:

1. The sensitivity and specificity of CTC for detecting lesions in the size range from 5 to 10 mm: CTC sensitivity and specificity for lesions above 10 mm in diameter are generally well over 90% – about as good as those for optical colonoscopy. There is evidence that a well designed CTC screening program can achieve at least 90% sensitivity and specificity in the size category from 7 and 10 mm, but not all studies have achieved this.

2. The use of non-cathartic pre-examination bowel preparation regimens: In general it may be less the invasive nature of conventional colonoscopy that results in poor compliance, but more the necessity for cathartic bowel preparation. CTC offers the potential for non-cathartic bowel preparation, through the use of barium or iodinated tagging agents, which impart a high-density to both stool and residual fluid, allowing increased contrast with soft-tissue polyps. Recent results with non-cathartic CTC have been very encouraging.

It seems likely that these issues will be resolved in the next few years. If CTC were to become a standard screening tool for the over 50s, the potential “market” in the US will soon be over 100 million people. Even if the recommended CTC frequency were to be that currently recommended for optical colonoscopy (every decade), this would imply that several million CTC scans might be performed each year. Should the relative simplicity of the CTC tests result in the recommended examination frequency being increased, then several tens of millions of these CTC scans might be expected to be performed in the US each year. It is pertinent, therefore to consider the radiation exposure and any potential radiation risk to the population from such a mass screening program.

Organ doses from CTC examinations can be estimated with standard techniques. These doses can be applied to organ- and dose-specific radiation cancer risk estimates, to estimate the excess cancer risk resulting from the radiation from a paired CTC exam.

Table I shows estimated organ doses for one of the more common CT scanners (GE LightSpeed Ultra). The scanner parameters were taken from a recent Mayo Clinic study by Johnson and colleagues, and are towards the low-dose end of published CTC protocols.

It can be seen from Table I that typical organ doses are less than 20 mSv, even for organs directly in the x-ray beam such as the colon, stomach, bladder, and kidneys. The sub-cohort of A-bomb survivors that received comparable radiation doses (A-bomb dose range 5–50 mSv, mean 20 mSv) does show a slight increase in cancer mortality compared to the control population, but this increase is of marginal statistical significance (p=0.15). It is also pertinent to point out that this A-bomb subcohort consists of individuals covering all age groups, and thus it is reasonable to assert that there is no direct statistically-significant evidence from A-bomb
Table I. Typical organ doses, background lifetime cancer risks, and additional absolute lifetime cancer risks from a paired CTC examination, on a 50 year old.

<table>
<thead>
<tr>
<th>Organ dose from paired CTC scans* (mSv)</th>
<th>Background organ-specific lifetime cancer risk (%)</th>
<th>Estimated additional absolute lifetime cancer risk due to paired CTC scans (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon (male)</td>
<td>13.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Colon (female)</td>
<td>13.2</td>
<td>4.8</td>
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<td>Bladder (male)</td>
<td>16</td>
<td>3.7</td>
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<td>16</td>
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<td>1.2</td>
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<td>Stomach (female)</td>
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<td>0.7</td>
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<td>Kidney (female)</td>
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<td>Lung (male)</td>
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<tr>
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<tr>
<td>Total (female)</td>
<td>32.9</td>
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</tbody>
</table>

* Paired CTC examinations with GE LightSpeed Ultra CT scanner, 130 mA, 120 kVp, 0.5 s rotation time, collimation 8×1.25 mm, pitch 1.35.

b Background organ-specific cancer risks for individual aged 60 (50 + 10 yr latency).

Table I shows the estimated absolute lifetime cancers risks, associated with the radiation exposure from paired CTC scans in a 50 year old. For comparison, the lifetime background cancer risks are also shown. As expected, the main organs at risk are the colon, stomach and bladder, as well as a risk for developing leukemic cancers. All the estimated absolute radiation risks are relatively small, the largest being less than 0.05% (1 in 2,000). Summed over all the organs at risk, the estimated absolute lifetime risk of cancer induction from a pair of CTC scans (with the scanner parameters from Table I) in a 50 year old is about 0.14%, about 1 in 700. Estimated risks for cancer mortality would, of course, be considerably less.

Several points need to be born in mind in relation to the estimated risks in Table I:

1. The risks are highly dependent on the scanner settings used, particularly the mAs and the pitch. The settings used in Table 1 are on the low-dose side of those used in current reported studies, but there is good evidence to suggest that the mAs and thus the dose could be decreased further by at least a factor of 5 (and perhaps as much as a factor of 10) from these settings, while still maintaining sensitivity and specificity for polyps larger than ~5 mm.

2. The estimated absolute cancer risks are highly age dependent. Thus, for example, the estimated radiation-associated absolute lifetime risk for colon cancer induction decreases from 0.044% for a CTC scan at age 50 (see Table I), to 0.022% for a scan at age 70.

3. If individuals receive multiple CTC screenings over a period of years, the radiation dose will, of course, increase proportionately. The most likely case is that any radiation risks will also increase proportionately. Specifically, at high doses, theory, animal data, and epidemiological data, suggest that fractionating a radiation exposure decreases the overall risk at a given dose, but at the low doses of relevance here, both theory and animal data suggest that the risks are roughly independent of fractionation.

4. There are quantifiable uncertainties involved in these radiation risk estimates. The largest is the uncertainties in “transferring” risk estimates from a Japanese population to a US population, but there are also uncertainties associated with the extrapolation of risks from somewhat higher doses, where the risks are statistically significant, and uncertainties associated with the reconstructed dosimetry estimates at Hiroshima and Nagasaki. Based on Monte-Carlo simulations of the various uncertainties, the upper and lower 90% confidence limits of the radiation risk estimates are about a factor of 3 higher and lower, respectively, than the point estimates.

In conclusion, there is persuasive evidence that a) colonoscopy-driven polypectomy can result in a significantly decreased incidence of colorectal cancer, and b) there is poor compliance with current guidelines for colorectal cancer screening. CT colonography (CTC), particularly using non-cathartic or minimally-cathartic techniques, has the clear potential to increase compliance. It is pertinent to note that, should non-cathartic CTC result in a significant increase in colorectal screening compliance, the overall colonoscopy demand would probably not change greatly, the decrease in the number of screening colonoscopies being compensated for by the increased demand for polypectomies of CTC-discovered polyps.

Because the geometry for CTC is highly advantageous (soft-tissue polyps projecting into an air or CO2 filled lumen), it can be performed using lower radiation doses than almost any other CT examination.

The cancer risks associated with the radiation exposure from CTC are unlikely to be zero, but they are small. A best estimate for the absolute lifetime cancer risk associated with the radiation exposure using typical current scanner techniques is about 0.14% for paired CTC scans for a 50 year old, and about half that for a 70 year old. These values could probably be reduced by factors of 5, or even 10, with optimized protocols.

Thus it seems clear that, in terms of the radiation exposure, the benefit-risk ratio is potentially large for CTC.
Cancer Risks after High Doses of Ionizing Radiation

David J. Brenner and Rainer K. Sachs

High-dose radiotherapy is being used with great success on a large number of cancer patients who may well live for many years post radiotherapy. The ten-year relative survival rates for prostate cancer and breast cancer in the U.S. are now about 76%, so there is increasing concern about the possibility of second-cancers in long-term radiotherapy survivors. Epidemiological studies of second cancers are, by their nature, limited to radiotherapy techniques that were common several decades ago. Thus it is important to be able to predict the carcinogenic effects of high doses of ionizing radiation, particularly on an organ-by-organ basis.

It has generally been assumed that cancer induction decreases rapidly at higher doses due to cell killing, in that dead cells cannot give rise to a malignancy and cell survival decreases exponentially or even faster with increasing dose. A standard quantitative implementation which describes a balance solely between carcinogenic alteration and cell killing is reproduced in most radiation biology textbooks, though with little epidemiological data in support. This standard high-dose model has frequently been used to analyze data on radiation-induced carcinogenesis.

However, several recent studies of radiation-induced second cancers in Hodgkin’s disease patients, which covered a very wide range of radiation doses and considered dose at the specific location of a second cancer, show that this standard high-dose picture is unrealistic, in that neither breast-cancer nor lung-cancer risks decreased with increasing dose, over the range from about 3 to 40 Gy; these data are shown in Figure 1. Also shown are corresponding cancer

Fig. 1. Excess relative risks for radiation-induced lung cancer (A) and female breast cancer (B). The data points from A-bomb survivors\(^4\,5\) are at moderate doses (<4 Gy). The data points at high doses are from studies of second cancers after radiotherapy of Hodgkin’s disease patients: lung cancer, median age at exposure: 45, median age at second cancer: 58; breast cancer, median age at exposure: 23, median age at second cancer: 42.\(^1\,2\) In the published reports the breast-cancer risks for Hodgkin’s disease patients were internally normalized to the lowest dose group (mean dose 3.2 Gy for Travis et al.,\(^1\) and 3.6 Gy for van Leeuwen et al.\(^2\)); these breast-cancer data (panel B) have here been renormalized based on the estimated A-bomb excess relative risks for breast cancer\(^4\) at 3.2 and 3.6 Gy respectively, adjusted for the different demographics and background risks of the Hodgkin’s breast-cancer patients vs. the A-bomb survivors. The data points for lung cancer (panel B) are taken directly from the published data.\(^3\) The dashed curves in each panel represent fits to the A-bomb data using the standard model\(^8\,9\) which involves a balance solely between induction of pre-malignant cells and cell killing; it is clear that the predictions of this model are quite inconsistent with the high-dose data.

\(^1\) University of California, Berkeley.
incidence data from A-bomb survivors, indicating the lower-dose region for which cancer risks are comparatively well known. The curves in Figure 1 show the standard quantitative model, discussed above, fitted to the A-bomb cancer-incidence data. The standard model, incorporating competition solely between carcinogenic and cell killing effects, is clearly inconsistent with the recent high-dose second-cancer data.

A likely resolution of this disagreement comes from the fact that repopulation of normal tissue is known to occur during and after fractionated high-dose exposure. Such repopulation tends to counteract cell killing, and thus might account for the gross discrepancies between the standard model and the new data illustrated in Figure 1. We therefore summarize here a systematic, biologically based, quantitative model for the dose dependence of radiation-induced cancer risks, emphasizing fractionated exposures, such as commonly used in radiotherapy. The model incorporates carcinogenic effects, cell killing effects and, additionally, cell proliferation/repopulation effects, using a minimum of adjustable parameters. We use this model to analyze and predict dose-response data for second-cancer induction at high radiation doses.

The model tracks the fate of an organ’s stem cells – pluripotent cells that are capable of regenerating normal tissue, but are also the primary cells at risk for a radiation-induced event that can eventually lead to cancer. We assume 1) that radiation increases the number of stem cells in the organ which undergoes a rate limiting step on the path to carcinogenesis (i.e., become “pre-malignant”); 2) that radiation-induced cell killing has a standard, well-understood dose-dependence; and 3) that during and shortly after radiotherapy the organ’s stem cells respond to radiation-induced cell killing exposure through accelerated repopulation which, under feedback control, tends to homeostatically restore their number back to their original steady-state number. This approach emphasizes biological processes during the period, lasting a number of weeks, from the start of radiation exposure until the relevant organ has repopulated. Subsequent carcinogenesis steps occurring on a substantially longer time scale are not analyzed explicitly, in that they are not expected to change the shape of the dose-risk relations, but are implicitly considered in the appropriate proportionality factor, discussed below, relating the yield of pre-malignant cells to the excess relative risk for the population of interest.

A simplified model (dashed lines in Fig. 2) requires no parameters except those adjusted from the A-bomb survivor data for the different demographics and background risks of the population of interest vs. the A-bomb survivors. Based on the analyses here for breast and lung, this simplified model should produce risk estimates good to within factors of 2 or 3 at least – an enormous improvement on the standard model whose predictions are shown in Figure 1.

A more general model (solid lines in Fig. 2) can provide a somewhat better description of the high-dose data, at the cost of requiring knowledge of extra parameters describing radiation-induced accelerated repopulation. These parameters need to be estimated from high-dose data; for example, recent studies of second cancers following radiotherapy for testicular cancer provide usable data at two high-dose points for stomach, small intestine, rectum, liver, gallbladder, pancreas, kidney, bladder, and acute leukemias.

As an example of the importance of understanding the shape of the dose-response relation at high doses, a model such as the standard model shown in Figure 1 would imply that organs very close to the tumor, which typically receive doses >20 Gy, would be at very low risk for radiation-
induced second-cancers. By contrast, the data and the models discussed here would suggest that organs closest to the tumor would be at the highest risk – which is clinically observed to be the case. These new models should also facilitate estimates of second-cancer risks after new types of high-dose radiotherapy, such as intensity-modulated radiotherapy.

References


Small Group Apprenticeship Program in Radiation Biology

Alan W. Bigelow and David J. Brenner

We have initiated a small-group apprenticeship (SGA) summer program for secondary-level students, as a method to facilitate the educational continuum between high school and college-level science, providing real-life experience working with high-tech equipment. The program will be run each summer at the Radiological Research Accelerator Facility (RARAF), where fundamental investigations into the radiobiological effects on mammalian cells are conducted through a broad beam and through controlled single-particle single-cell microbeam irradiation.

Sessions are held twice a week for 6 weeks, for a total of 48 contact hours. The instructors are drawn from the staff at RARAF – a mix of technicians, post docs, lab managers, and professors – many of whom are experienced teachers at the university level. While we teach some specific skills, the course design focuses very much on research training, career exploration, and mentoring. The course structure first features a number of presentations and demonstrations from the RARAF staff to the small group. The remainder of the course emphasizes the individual student projects, where final presentations are given on the last day of the course.

For the SGA program at RARAF, we invite up to six students to participate in multidisciplinary studies. Our radiation biology laboratory is a multidisciplinary facility; hence, the apprenticeship program follows that example and is designed for students to receive training in more than one discipline. Student apprentices are enriched with a global view of the science activities at RARAF, where we can accommodate three students with a biology background and three students with a physics background.

More specifically, the activities at RARAF are shared between cell biology and a physics-based infrastructure for a variety of cell irradiations: single-cell targeting using the microbeam and broad-beam irradiation using the track segment facility, for instance. The merge between these fields occurs regularly at RARAF, where the physics activities have the cell-irradiation experiments as their goal and where the biology experiments hinge on the use of a 4 MV Van de Graaff particle accelerator. In addition, there are important support projects based in engineering, optics, computer simulation and computer programming. The following is a list of the student project titles, with the student name and the name of the mentor:

- Designing a Mirau Immersion Lens; Chun Che Peng, Greg Ross.
- To Determine the Signal Transduction Pathways Responsible for the Bystander Effect; Lusana Ahsan, Brian Ponnaiya.
- Testing for the Presence of Various Stress Proteins to Show the Presence of the Bystander Effect; Perry Leung, Brian Ponnaiya.
- Laser Ion Source; Ross Kelly, Alan Bigelow.
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Stephen Mitchell, Ph.D. – Post-Doctoral Research Scientist
Giuseppe Schettino, Ph.D. – Post-Doctoral Research Scientist
Gloria Jenkins-Baker, B.A. – Biology Technician

This is a major year for RARAF. Our Van de Graaff accelerator, which is 55 years old and has provided us with charged particle beams for over 38 years, will be decommissioned this May. We will install a new Singletron from High Voltage Engineering (HVE) that should provide us with increased voltage, stability and beam current.

Among the major accomplishments this year:

- The first microbeam irradiations using the new microbeam facility (Microbeam II);
- Construction of a successful quadrupole triplet, producing a helium ion beam spot 3.5 µm in diameter;
- Construction of the stand-alone microbeam vacuum system and installation and testing of the quadrupole magnets.

Research Using RARAF

For several years, the focus of most of the biology experiments at RARAF has been the “bystander” effect, in which cells that are not irradiated show a response to radiation when in close contact with or even only in the presence of irradiated cells. Several experiments examining this effect were continued this year and new ones initiated, observing a variety of endpoints to determine the size of the effect and the mechanism(s) by which it is transmitted. Evidence has been obtained for both direct gap junction communication through cell membrane contact and indirect, long-range communication through media transfer. In some experiments, the unirradiated cells can be identified due to differential staining and scored directly, in other experiments unirradiated cells are physically separated from the irradiated cells during irradiation. Both the microbeam and the track segment facilities continue to be utilized in various investigations of this phenomenon. The single-particle microbeam facility provides precise control of the number and location of particles so that irradiated and bystander cells may be distinguished but is somewhat limited in the number of cells that can be irradiated. The track segment facility provides broad beam irradiation that provides a random pattern of charged particles but allows large numbers of cells to be irradiated and multiple users in a single day.

In Table I are listed the experiments performed at RARAF from November 1, 2003 through October 31, 2004 and the number of days each was run in this period. Use of the accelerator for experiments was 50% of the normal available time, 50% higher than last year and the highest we have attained at Nevis Labs. In addition, for the first time in over a decade outside users accounted for half the experiment time. Sixteen different experiments were run during this period, about the same as the average for 1997–2003.

Seven experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH) and the Department of Energy (DOE). Nine experiments were performed by outside users, supported by grants and awards from the NIH, the National Aeronautics and Space Administration (NASA), the National Science Foundation (NSF), and the Ministry of Education, Science, Sports and Culture of Japan. Brief descriptions of these experiments follow.

Eric Hall and Stephen Mitchell of the Center for Radiological Research (CRR) continued investigations involving the oncogenic neoplastic transformation of mouse C3H 10T½ cells (Exp. 73). Using the microbeam facility, 10% of the cells were irradiated through the nucleus with 2 to 12 helium ions. Cells were plated at densities of approximately 200 and 2000 per dish to try to observe the relative contribution of cell-cell communication to the bystander effect. Cell killing and transformation were greater for the cells plated at the higher density relative to those plated at the lower density. The results imply that gap junction communication has a greater role in the bystander effect than media transfer. In another aspect of the study, a novel radiation apparatus where irradiated and non-irradiated cells were grown in close proximity was used to investigate the relationship between the bystander effect and adaptive response in C3H 10T½ cells. Special “strip” track segment dishes were made by cutting the Mylar surface on the bottom of special cell dishes into many equal strips and removing alternate strips. The remaining Mylar strips are sufficiently thick to stop the incident ions, so that cells plated on these surfaces are not irradiated. These dishes were placed inside standard track segment dishes that have a complete Mylar surface 6 µm thick, through which the ions readily pass. Cells are plated on the combined surface. When the cells were left in situ for 24 h for the non-hit cells to co-culture with cells irradiated with 5 Gy of α-particles using the track segment facility, a significant increase in both cell killing and oncogenic transformation frequency was observed. If these cells were treated with 2 cGy of x-rays 5 h prior to co-culture with irradiated cells, approximately 95% of the bystander effect was canceled out. A 2.5-fold decrease in the oncogenic transformation frequency was also observed. To investigate whether mouse embryo fibroblast cells haplo-insufficient for one or more of a number of genes of known importance, namely ATM, BRCA1 and RAD9, are radiosensitive to cell lethality and/or oncogenic transformation, cells that are heterozygous for these genes were irradiated on the track segment facility. To date no difference has been seen for survival, whereas cells haplo-insufficient for both ATM and RAD9 are signifi-
Table I. Experiments Run at RARAF, Nov. 1, 2003–Oct. 31, 2004

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Experimenter</th>
<th>Institution</th>
<th>Exp. Type</th>
<th>Title of Experiment</th>
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<td>73</td>
<td>S. Mitchell, E.J. Hall</td>
<td>CRR</td>
<td>Biology</td>
<td>Neoplastic transformation of mouse cells by α-particles</td>
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<td>R. Eliassi, G. Garty</td>
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<td>R. H. Maurer, et al.</td>
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<td>Physics</td>
<td>2. Calibration of a portable real-time neutron spectrometry system</td>
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<td>S. Amundson</td>
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<td>B. Ponniaya, C.R. Geard</td>
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<td>Biology</td>
<td>Single cell responses in hit and bystander cells: single-cell RT-PCR and protein immunofluorescence</td>
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<td>G. Jenkins, C.R. Geard</td>
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<td>Damage induction and characterization in known hit versus non-hit human cells</td>
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<td>M. Suzuki (H. Zhou)</td>
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<td>Chromatid fragment induction detected with the PCR technique by cytoplasmic irradiation in normal human bronchial cells</td>
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<td>W. Morgan (S. Mitchell)</td>
<td>University of Maryland</td>
<td>Biology</td>
<td>Investigation of the bystander effect</td>
<td>6.1</td>
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</table>

Note: Names in parentheses are CRR members who collaborated with outside experimenters.

Development of a method to detect explosives in baggage (Exp. 82) was resumed this year. Ravash Eliassi, an undergraduate student at UCLA, in collaboration with Guy Garty and under the guidance of Gerhard Randers-Pehrson, both of the CRR, made measurements of neutron spectra and yield from the Be(7p,n) reaction using a very thin beryllium target. The detection system is based on resonant scattering of 0.43 MeV neutrons by nitrogen and oxygen. Measurements were made for several combinations of reaction angle and incident proton energy that produce 0.43 MeV neutrons to determine parameters producing the highest yield and the spectrum that contains the highest percentage of neutrons of the desired energy.

Richard Maurer, David Roth and James Kinnison of Johns Hopkins University performed an irradiation (Exp. 89) with neutrons of a pair of charged-coupled devices (CCDs) to be used to send video signals from the NASA New Horizons probe that will travel past Pluto to the outer asteroid belt. Since the probe will spend much of its life too far away to use solar panels, there will be a radioisotope thermoelectric generator (RTG) on board that uses the radioactive decay of plutonium to produce electricity. The potential effect of neutrons emitted by the plutonium on the resolution of the CCDs was determined. A neutron energy of 2.1 MeV was used because it is near the average energy of the neutrons produced by the fission of plutonium.

Sally Amundson, now a member of the CRR, is conducting two types of experiments concerning the radiation-induced gene expression profiles in human cell lines using cDNA microarray hybridization and other methods (Exp. 92). The first involves track segment irradiation for comparison of gene expression responses to direct and bystander irradiation. In these experiments, gene expression at 4 and 24 hours post treatment are compared. Early experiments have worked well and they are now being repeated to establish reproducibility and to obtain sufficient data to begin an analysis. The second type of irradiation involves use of the microbeam to irradiate either cell nuclei or cytoplasm. These experiments require optimization and validation of cDNA amplification techniques to produce sufficient material for microarray hybridization from the small number of cells irradiated. Initial results indicate the system is robust and accurate. RNA from single microbeam dishes has been isolated successfully and early amplification and hybridization results are highly encouraging. Gene expression profiles have been obtained from both nuclear and cytoplasmic irradiation at 4 and 24 hours after treatment. As in the case of the track-segment bystander studies, these experiments must still be repeated to obtain reproducible data that can be analyzed to reveal gene expression trends.

Two studies investigating the bystander effect were continued by Brian Ponniaya and Charles Geard of the CRR. In one study (Exp. 94), levels of p21 production were measured in individual normal human fibroblasts using immunofluo-
rescent staining. This procedure permits observation of the variation in response of individual cells to radiation instead of just the average response of a large number of cells. From 1 to 100% of the cell nuclei were irradiated with helium ions using the microbeam facility. The second investigation uses the track segment facility for broad-beam charged particle irradiations of human fibroblasts and epithelial cells immortalized with telomerase (Exp. 106). Special cell dishes are made from stainless steel rings with thin Mylar windows glued on both sides onto which cells are plated, eliminating any possibility of cell-cell contact between cells on opposing surfaces. The dish volume is filled with medium. Cells on one surface are irradiated with \(^3\)He ions; cells on the opposite surface are unirradiated because the particles stop in the medium before reaching them. They have used this novel co-culturing protocol previously to demonstrate bystander responses observed by the induction of micronuclei and chromosomal aberrations in non-irradiated normal human fibroblasts following irradiation with helium ions using the track segment facility. These studies have since been expanded to include analyses of cellular signaling pathways in both irradiated and bystander cells at both the protein and mRNA levels. Cells were observed in situ after irradiation with doses from 0.05 to 1.6 Gy of 12 keV/\(\mu\)m helium ions or 0.01 to 0.10 Gy of 12 keV/\(\mu\)m protons. The proteins examined by immunofluorescence techniques included p21/WAF1 and members of the MAP kinase signaling pathway, i.e., ERK1/2, p38 and pJNK, whose phosphorylation status has been shown to be altered in both irradiated and bystander cells. Levels of mRNA from early response genes, including \(c-fos\), \(c-jun\), \(junB\) and p21/WAF1 were also assayed using RT-PCR protocols.

Charles Geard and Gloria Jenkins of the CRR continued their studies of the bystander effect in several cell lines using the microbeam facility (Exp. 103). Normal human fibroblasts and human mammary epithelial cells were irradiated with helium ions, targeting 1%, 10% and 100% of the cell nuclei. Endpoints for various experiments included micronucleus production in S phase, production of p21, p53 and H2AX in the fibroblasts and production of H2AX in the mammary cells. In some of the experiments the bystander cells were stained with a dye different than the irradiated cells so the two could be distinguished.

Hongning Zhou and Tom Hei of the CRR continued to use the single-particle microbeam facility to try to identify the signaling transduction pathways involved in radiation-induced bystander mutagenesis (Exp. 110). Functional deficiency cell lines or cells treated with inhibitors are irradiated using the microbeam facility. A fraction of the cells is irradiated with alpha-particles. The cells are kept in situ for 2, 6, 24 or 48 hours after irradiation, thereby increasing the number of cells and the time for interaction. In addition, some experiments have been performed using the track segment facility using the “strip” dishes described for Experiment 73. The mRNA extracted from the cells is analyzed using microarrays. Preliminary data show some changes in gene expression in the bystander cells.

The Department of Defense is interested in the biological effects of depleted uranium (DU), especially after its signifi-
Development of Facilities

Our development effort has somewhat decreased this year from last but is still very high. We have added another person to the development team: Giuseppe Schettino, who developed the x-ray microbeam for the Gray Cancer Institute.

Development continued or was initiated on the microbeam facilities and a number of extensions of their capabilities:
- Development of focused accelerator microbeams
- Source-based microbeam
- Focused x-ray microbeam
- Precision z-motion stage
- Laser ion source
- Secondary emission ion microscope (SEIM) for viewing focused beam spots
- Non-scattering particle detector
- Advanced imaging systems
- New accelerator

Development of focused accelerator microbeams

A quadruplet lens with titanium-coated rods was mounted in the alignment tube for the double lens system and placed in the beam line for the new microbeam facility. It focused the beam to less than a 7 µm diameter. Measurements made of the voltages required to obtain various beam spot geometries when all and only some of the lens elements were used provided data for our consultant at the University of Louisiana, Alexander Dymnikov, to calculate parameters for the double quadrupole triplet lens assembly that will be used to focus the ion beam to a diameter of 0.5 µm.

The first quadrupole triplet based on these calculations has been constructed, placed in a separate alignment tube and inserted in the beam line in place of the quadruplet lens. This triplet lens has produced a beam spot for helium ions 3.5 µm in diameter. It required very little voltage conditioning, produced an acceptable beam in less than a week of adjustments and has been used for microbeam irradiations for several months. The second quadrupole triplet is under construction in our shop and will be inserted for testing in place of the present one, once it is completed. When the voltages on this second lens have been adjusted to produce the smallest beam spot attainable, the two lenses will be mounted in a single tube for testing of the compound lens system that will produce a sub-micron beam spot.

Source-based microbeam

A stand-alone microbeam (SAM) has been designed based on a small, relatively low activity radioactive alpha-particle emitter (5 mCi ²¹⁰Po) plated on the tip of a 1-mm diameter wire. Alpha-particles emitted from the source will be focused into a spot 10 µm in diameter using a compound quadrupole lens made from commercially available permanent magnets, since only a single type and energy of particle will be focused. The pair of quadrupole triplets is similar to the one designed for the sub-micron microbeam, the only difference being that it uses magnetic lenses, rather than electrostatic lenses. A small stepping motor rotating a disc with holes will be placed just above the source to chop the beam, enabling single particle irradiations. The end station for the original microbeam will be used to perform microbeam irradiations. The SAM will replace the accelerator-based system in our original microbeam laboratory and can be used during the period when the Van de Graaff is being removed and the Singleton installed.
The original microbeam irradiation station moved to the floor above the original microbeam room (normally storage) for use in testing the stand alone microbeam system. One of the permanent magnet quadrupole triplets is inside the white enclosure (to keep it clean) in the middle of the table on the left.

The magnets have been received and mounted in the support structure manufactured in our shop. To test the system and adjust the lenses, we are using a helium beam from the accelerator on a thin aluminum foil to produce an energy and energy spread that match those calculated for the polonium source. The end station for our original microbeam was moved to the floor above because additional room for the lens structure was required between the table and the bending magnet. The first lens was adjusted to provide a good line image on a CCD mounted at the focal point of the lens. By adjusting the second lens, the beam has been focused to a spot 30 µm in diameter using the compound lens system. Further adjustments of the lenses are expected to result in a beam spot 10 µm in diameter.

A glove box has been purchased in which to plate the polonium on the wire to make the alpha source. This procedure is expected to be quite simple and exhaust all the polonium from the solution. A thin layer of gold will be plated over the source to contain the polonium. We are awaiting an amendment to our radioactive materials license so we can receive a small quantity of polonium as a test of the procedure.

**Focused x-ray microbeam**

We have investigated expanding the microbeam repertoire to include soft x-rays (Al $k_{\alpha}$, 1.49 keV). Microbeam studies with focused high-energy x-rays or gamma-rays are not feasible due to Compton scattering effects, so we are limited to x-ray energies where the predominant mode of interaction is photo-electron absorption. A proton beam will be focused onto an aluminum foil using the compound electrostatic lens. The characteristic x-rays produced in the foil then will be focused to a diameter of 1 µm using a zone plate with a focal length of 12.7 mm. Calculations performed indicate that a 1 nA proton beam should produce a dose rate of 0.1 Gy/sec of x-rays, adequate for the biological studies envisioned. An alternative system is being investigated in which a capillary tube with an inside diameter of a few microns would be used instead of a zone plate to collimate or focus the x-rays. The end of the microbeam line will be modified so that the target and focusing system can be rotated into or out of the beam path to change irradiation modalities quickly and without interrupting the vacuum system.

**Precision z-motion stage**

The high-precision stage from Mad City Labs in Wisconsin that also has a vertical motion is in routine use in the Microbeam II irradiation station. This stage has a range of motion of 200 µm in the x and y directions and 100 µm in the z direction, with nanometer positioning. Because of its limited range of motion in the horizontal plane, it has been mounted within a coarser stage in order to be able to access the entire area on which cells are plated. The vertical motion is required for the imaging techniques described below. The stage is also used to raise and lower the sample over the exit window during movement to minimize the separation from the window and thereby reduce beam spread due to scattering in the window.

**Laser ion source**

Development of the laser ion source continues to progress. The mounts for the mirrors and lenses to direct and focus the laser beam on the target have been constructed and installed and testing of the system has begun. The motor system advances the target surface along a spiral with each laser pulse in order to obtain a fresh surface and maintain yield. Methods are being examined to protect the focusing lens from material ejected by the target and to maintain the laser focus to prevent damage to the target.

The terminal of the new Singletron has been designed to accommodate the ion source without any modification to our present design. We have decided not to install the source in
Secondary emission ion microscope

As we improve the spatial characteristics of the microbeam system, it becomes increasingly important to be able to assess the beam quality in order to adjust the system to its optimum capabilities. A secondary electron ion microscope (SEIM) has been designed and is currently being constructed. This device will enable us to measure the beam profile and position in real time with sub-micron resolution and sensitivity to single ions (1–5 MeV protons, as well as heavier ions). The SEIM design was inspired by the technique of photoelectron microscopy (PEM) and we gratefully acknowledge the advice of a world expert in PEM, Dr. Gertrude Rempfer, in finalizing our design. The SEIM is based on secondary electron emission (SEE) by a film on which the ions in the beam are incident. The ejected electrons are focused to form a magnified image on an image-intensified CCD. In order to overcome the chromatic and spherical aberrations inherent in the electrostatic lens and provide a more compact instrument, the electrons are bent by a 45º angle, reflected by an electrostatic mirror and bent by an additional 45º before reaching the detector. This “folded” design of the SEIM is a novel one, developed at RARAF. Calculations indicate a magnification of ~500 can be achieved, yielding a resolution of 0.1–0.2 µm.

We have built an “unfolded” SEIM, consisting of the electrostatic lens and the electron detector but without the magnet and mirror, in order to test the lens properties. For this version, simulations have shown that both the resolution and magnification are 10–20 times inferior to the folded SEIM. For testing and calibration purposes the SEE foil was replaced with a quartz window on which a micron scale pattern of aluminum was evaporated. The pattern was illuminated with low intensity UV light and the resulting photoelectrons were imaged, similar to a photoelectron microscope. In a sample image based on ~200,000 electrons the width of the spot edge allows us to estimate the SEIM resolution at 4.3 µm RMS, in good agreement with the prediction of 4–5 µm made by simulations. The predicted magnification (16x) is also in good agreement with the measured value of 20x.

The SAM would also be useful for groups that desire to perform microbeam experiments at their home institutions but lack an appropriate accelerator. It is estimated that a complete SAM system, including the microscope, could be built for ~$100k.

Non-scattering particle detector

To irradiate thick samples, such as model tissue systems or oocytes, or to use particles with very short ranges, such as the heavy ions from the laser ion source, a completely non-scattering upstream particle detector is necessary. A novel particle detector has been designed on the basis of a long series of inductive cells coupled together into a delay line. The Lumped Delay Line Detector (LD2) will consist of 300 silver cylinders 3 mm long with a 2.2 mm inside diameter connected by inductors and capacitively coupled to ground. The cylinders are glued to a semi-cylindrical tube of dielectric material 1 m long for mechanical support. The dielectric has a semi-cylindrical metal tube around it that can be rotated about its axis to adjust the capacitance. If the individual segment delays are set (by adjustment of the capacitance) such that the propagation velocity of the pulse equals the projectile velocity, the pulses induced in all segments will add coherently, giving a fast electron pulse at one end of the delay line that is 150 times larger than the charge induced on a single cylinder. This easily detectable charge of at least 150 electrons will be amplified to provide the detection pulse for the particle counter. The surface-mount inductors have been purchased. The silver cylinders originally purchased proved to be too eccentric and badly finished. Silver tubing has been purchased from which our shop will machine the cylinders. The rest of the detector parts have been designed and await machining. Testing of the detector will probably not begin until we have the new accelerator operational. It is anticipated that this detector will become the standard detector for all the irradiations on the new microbeam facility.

Advanced imaging systems

Development continued on new imaging techniques to view cells without using stain and to obtain three-dimensional images of unstained cells. Two different techniques are being investigated: phase-shifted interference microscopy and quantitative non-interference phase microscopy (QPM).

In phase-shifted interferometry images are obtained with an immersion Mirau interferometric objective in a sequence of three sub-wavelength path differences (phase shifts) between the sample and the lens. For this technique, it is important that the substrate for the cells be optically flat. The combined images can be used to produce a topographic image by solving for the phase shifts at each point. The essence of the algorithm for determining these phase shifts is to solve for three variables with an over-determined system of four equations. Results so far are encouraging. It has not yet been determined whether the cells will have to be on a reflective surface. The Mirau lens has been purchased and the immersion system has been designed, with assistance from Chun-Che Peng, one of the high school students.

The other method being investigated is a relatively new technique that can generate phase images and phase-amplitude images using a standard microscope. To obtain a quantitative phase image, an in-focus image and very slightly positively and negatively defocused images are collected. The resulting data can be used to yield the phase distribution by Fourier-transform methods. Test images sent to the software manufacturer yielded surprisingly good resultant images. We are evaluating a trial copy of the Fourier transform-based software for generating phase images or phase-amplitude images from the three microscope images.

Both of these techniques require rapid automated motion in the X-Y plane for locating the cells as well as in Z for changing the focal plane. In the case of immersion-based Mirau interferometry, the precision must be on the order of...
tens of nanometers. The Mad City stage will be able to provide the vertical motion required by both these methods to obtain the necessary images at different distances between the sample and the lens.

A method of identifying the stage of the cell cycle using the microbeam image analysis system is being investigated. The Hoechst 33342 dye used to stain cell nuclei for identification in the microbeam irradiation system binds to the DNA in the nucleus. Cells in G2 have twice the DNA of cells in G0 or G1, with cells in S phase increasing from the level in G1 to that in G2. Consequently, it seems reasonable to believe that the amount of stain in a nucleus could be used to indicate which of these phases a cell is in. Initial measurements using cells synchronized in G1 by serum starvation to indicate which of these phases a cell is in. Initial measurements using cells synchronized in G1 by serum starvation have proven inconclusive. Additional measurements are continuing.

**New Accelerator**

The specifications for the Singletron from HVE in general exceed those for the Van de Graaff it is replacing. The maximum terminal voltage is 5 MV with 100 V or less ripple at 3 MV. The maximum voltage ever attained by the Van de Graaff was 4.4 MV and the ripple was never less than 1-2 kV. The maximum beam currents are 200 µA of protons, 100 µA of deuterons and 1 µA of helium ions, similar to that of the Van de Graaff.

The Singletron is scheduled to be shipped by boat from the Netherlands about May 20 of this year and should arrive in port 1–2 weeks later. The Singletron will then be carried by truck to RARAF where it will be lifted by crane, placed on its rails and rolled into place. The Van de Graaff, of course, will have to be removed before then.

We will begin the process of disconnecting the Van de Graaff wiring and plumbing on May 2. All the old control wires and the racks that supply power to the accelerator will be removed. At the console, all the voltage regulation electronics and controls for the ion source and charging system also will be removed. The Singletron will be controlled by a computer at the console through a fiber-optic link to the accelerator rack. The accelerator vacuum system will be disconnected from the rest of the beam line and the interior of the Van de Graaff dismantled. The accelerator pressure vessel, the interior components and especially the base plate will be assayed to determine whether the pieces can be disposed of as regular scrap or will have to be handled as radioactive material. The interior of the acceleration tube will be tested for tritium contamination that may have occurred from years of using tritium targets to make neutrons, and decontaminated before disposal, if necessary.

Although the Van de Graaff was originally put in place using the overhead crane in the building, removal by the same method is no longer practical because of the shield blocks over the area and the labs that have been built on them, particularly the Microbeam II lab. Therefore, in order to remove the baseplate and pressure vessel, the rear wall of the building extension will be cut open and the accelerator pulled out onto a temporary platform where it will be lifted by crane onto a truck. Rails for the Singletron will be put in place on this platform temporarily so that the new accelerator can be rolled in through the same opening. We anticipate the entire process of removing the Van de Graaff and putting the Singletron into place will take about one month.

After the building has been restored and the electric and fiber-optic cables are installed, a representative from HVE will supervise the assembly of the Singletron by the RARAF staff. Once the accelerator is assembled, performance tests will be made to certify that the accelerator meets (or exceeds) the specifications HVE stated in their bid. HVE estimates that this process will take 4–5 months, so that there will be a 5–6 month period when no accelerator will be available.

**Accelerator Utilization and Operation**

Accelerator usage is summarized in Table II. The accelerator now is started at 7:30 AM on most days and run into the evening on many nights for experiments, development and repair. In addition, Dr. Aprile’s Astrophysics group (Exp. 123) has run continuously over a few weekends. This has resulted in a total use (117%, including repairs) that considerably exceeds the nominal accelerator availability of one 8-hour shift per weekday and is the highest we have had at Nevis Labs.

Use of the accelerator for radiobiology and associated dosimetry increased almost 50% over 2002–2003 and was about the same as the average for 1999 to 2003. About half the accelerator use for all experiments was for microbeam irradiations. Because of the relatively low number of cells that can be irradiated in a day, microbeam experiments usually require considerably more beam time than broad beam irradiations to obtain sufficient biological material, especially for low probability events such as transformation and mutation, and therefore normally constitute a large fraction of the experimental use.

Radiological physics utilization of the accelerator increased again this past year, primarily due to the calibration of the xenon detector (Exp. 123) that comprised about 18% of all the time used for experiments. On two weekends, this experiment ran continuously for 2½ days. As usual, there were no chemistry experiments this reporting period.

Use of the accelerator for online development declined about 15% over last year but still comprised over 40% of all available time. For several months, many more than the usual number of extra shifts was worked in the evening, on weekends and holidays.

Table II. Accelerator Use, Nov. 2003–Oct. 2004

<table>
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<th>Percent Usage of Available Days</th>
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<tr>
<td>Radiobiology and associated dosimetry</td>
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<tr>
<td>Radiological physics and chemistry</td>
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<td>On-line facility development and testing</td>
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Accelerator maintenance and repair time increased by 50% over last year, returning to the level of 2001–2002, and was also about 50% higher than the long-term average due to continued problems in the power supply in the terminal used to spray negative charge on the charging belt. Despite several modifications to the supply to reduce sparking, one of two strings of high voltage diodes in the supply would short out. We believe we now have located the cause of this problem and have repaired the power supply. The vacuum leak in one of the sections of the acceleration tube is a problem that has troubled us for several years, but at the moment is only an annoyance since a procedure has been developed to reseal the leak each time we open the accelerator for repair. No replacement of the section is planned because the accelerator will be dismantled in about 2 months to make room for the new one. No major repairs or modifications to the accelerator were performed. Once the new accelerator is installed, we anticipate much less accelerator maintenance, not only because the Singletron will be new, but also because it will be charged electronically (similar to a Cockroft-Walton) and will have few moving parts (no belt or chains). It has an RF ion source that also should require less maintenance than the Duoplasmatron source we are presently using. During the 5–6 months for the removal of the Van de Graaff and installation of the Singletron there will be no “on-line” development or accelerator-based experiments. All biology will be performed using the stand-alone microbeam. However, considerable development will continue since much of it is concerned with optical or other imaging issues and these don’t require an accelerator.

Training
This year we have had several students train at RARAF. During the summer of 2004, five students from Stuyvesant High School in Manhattan (Lusana Ahsan, Ross Kelly, Perry Leung, Deep Parikh, and Chun-Che Peng) spent at least two half days each week for 6 weeks working on projects in biology or physics that they selected. At the end of their projects, the students gave very professional presentations of their work. Their knowledge and commitment to their projects was impressive. This summer program for high school students now will be offered every year.

Ravash Eliassi, an undergraduate student from UCLA, spent ten weeks during the summer measuring the yields and neutron spectra produced by protons on a very thin beryllium target (Exp. 82). This type of target might be used to produce neutrons for the detection of explosives by resonant neutron scattering.

David Ross, an undergraduate student from the University of North Texas spent 4 weeks starting in December 2004 studying whether the phase of the cell cycle could be determined by the microbeam image analysis system using quantitative analysis of the Hoechst stain.

Personnel
The Director of RARAF is Dr. David Brenner. The Van de Graaff accelerator facility is operated by Mr. Stephen Marino and Dr. Gerhard Randers-Pehrson. Our ranks have now swelled to a total of seven physicists, an increase of two.

Dr. Alan Bigelow, now an Associate Research Scientist, is continuing the development of the laser ion source and an optical system for 3-dimensional viewing of cells.

Dr. Guy Garty, a Staff Associate, is working on the development of a stand-alone microbeam, the secondary emission ion microscope (SEIM) and an inductive detector (LD²) for single ions.

Mr. Greg Ross is a Programmer/Analyst, assisting with various programming tasks and working on the development of a stand alone microbeam and new methods of imaging cells.

Dr. Giuseppe Schettino, a Post-Doctoral Fellow, arrived in November from the Gray Lab in England. He will work primarily on the development of the x-ray microbeam.

Dr. Furu Zhan, a Post-Doctoral Fellow, returned to China in May, 2004.

Biologists from the Center for Radiological Research are stationed at the facility in order to perform experiments:

- Dr. Charles Geard, the Associate Director of the CRR, continues to spend most of each working day at RARAF. In addition to his own research, he is collaborates with some of the outside users on experiments using the single-particle microbeam facility.
- Dr. Brian Ponnaiya is an Associate Research Scientist performing experiments using the track segment and microbeam irradiation facilities.
- Ms. Gloria Jenkins, a Biology Technician, performs experiments on the microbeam facility for Dr. Geard.
- Dr. Stephen Mitchell, a Post-Doctoral Fellow, continues to perform research involving neoplastic transformation of cells.


7. Garty G, Randers-Pehrson G and Brenner DJ. Develop-


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The new RARAF stand-alone microbeam: a) Diagram of the stand-alone microbeam's principle elements. A small, high specific-activity α-emitter is used as a source. A compound magnetic lens, consisting of 24 permanent magnets arranged in two quadrupole triplets, focuses the emitted α-particles. The first triplet is placed 2 m above the source, with a second identical triplet placed 2 m above the focal plane of the first. The cells to be irradiated are placed at the image plane on a voice coil stage. The endstation consists of the voice coil stage and a microscope with a particle detector mounted on the objective lens. b) The microbeam endstation and immediately below it the upper magnetic quadrupole triplet. c) The source holder (near floor) and the lower quadrupole triplet. d) A system for electroplating polonium onto the end of a wire, to create the 1-mm diameter, α-particle source.
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Some views of the RSO staff at work and at leisure.

Tom Juchnewicz (left), with James Dolan (2nd from right), making a point during planning for decommissioning the $^{60}$Co teletherapy facility at NYPH Dept. of Radiation Oncology.

Smiling faces in the Radiation Safety Office (l-r): Raquel García, Jacob Kamen and Jennifer Curiel.

Roman Tarasyuk, surrounded by drums of radioactive material being held in the radioactive waste storage facility for decay/disposal or shipment with a vendor for removal.

Preparing for the holiday party (l-r): Dae In Kim, James Dolan and Mutian Zhang.
Dr. Hall and the RSO Staff (standing, l-r): Tom Juchnewicz, James Dolan, Dae In Kim, Roman Tarasyuk, Mutian Zhang, Salmen Loksen, Dr. Eric Hall, Bruce Emmer, Dr. Jacob Kamen and Ahmad Hatami; (sitting, l-r): Dong Michelle Kang, Yvette Acevedo, Milvia Perez, Raquel Garcia and Jennifer Curiel.

PROFESSIONAL STAFF

- Salmen Loksen, M.S., CHP, DABR; Director, Radiation Safety Officer
- Ahmad Hatami, M.S., DABR, DABMP; Assistant Director
- Thomas Juchnewicz, M.S., DABR; Assistant Radiation Safety Officer
- Jacob Kamen, Ph.D., NRRPT, CHP; Assistant Radiation Safety Officer
- Bruce Emmer, M.S., DABMP, DABR; Physicist
- Mutian Zhang, M.S.; Acting Radiation Protection Supervisor
- Dae In Kim, M.S.; Health Physicist
- Shuntong Guo, M.S., CHP; Junior Physicist
- James Dolan; Junior Physicist

TECHNICAL STAFF

- Olga Loukhton, M.S.; Chief Technician
- Roman Tarasyuk; Technician B
- Dong Michelle Kang, M.S.; Technician B

ADMINISTRATIVE AND SECRETARIAL STAFF

- Diana Morrison; Administrative Assistant, assigned to the JRSC
- Yvette Acevedo, A.A.S.; Administrative Aide
- Raquel Garcia; Clerk B
- Milvia Perez, A.A.S.; Clerk B
- Stephen Benson, B.A.; Administrative Assistant
- Jennifer Curiel, Administrative Staff
INTRODUCTION

On May 19, 1957, the President of Columbia University distributed a memo entitled “Directive to All University Departments Having a Source of Ionizing Radiation,” advising all parties of the expanded function of the Radiation Safety Committee.

Later, a notice entitled “Radiation Safety Guide for Columbia University,” dated February 10, 1959, named Philip M. Lorio as the Health Physics Officer for University Departments and Laboratories other than the College of Physician & Surgeons, where Dr. Edgar Watts was the named Health Physics Officer. The Chairman of the Radiation Safety Committee was Dr. Gioacchino Failla, who initiated the Radiological Research Laboratory in the Department of Radiology of Columbia University Medical Center (CUMC).

By agreement between The Presbyterian Hospital in the City of New York (PH) and Columbia University (CU), the Radiation Safety Office was established as an autonomous unit in 1962 for the purpose of maintaining radiation safety. The Joint Radiation Safety Committee (JRSC), appointed by the Medical Board of the Presbyterian Hospital in the City of New York and the Vice President for Health Sciences of Columbia University, is charged with the responsibility of defining and ensuring enforcement of proper safeguards in the use of sources of ionizing radiation.

Dr. Harald H. Rossi, Director of the Radiological Research Laboratories, was appointed Chairman of the Joint Radiation Safety Committee. Under his direction, this committee developed a “Radiation Safety Code and Guide,” the administration of which is assigned to the Radiation Safety Officer. Dr. Eric J. Hall, the present Director of the Center for Radiological Research, now chairs the JRSC.

The present Joint Radiation Safety Committee of the Columbia University Medical Center and the New York State Psychiatric Institute came into existence through an agreement made on February 12, 1991 between New York State Psychiatric Institute (NYSPI), the College of Physicians and Surgeons of Columbia University (P&S), and The Presbyterian Hospital in the City of New York (PH). This agreement combined several overlapping clinical and educational programs, including all programs for ensuring radiation safety. The current Director of the Radiation Safety Office and Radiation Safety Officer, Salmon Loken, CHP, DABR, was appointed on December 16, 1996.

The Radiation Safety Office reports to and advises the Joint Radiation Safety Committee of Columbia University Medical Center, New York Presbyterian Hospital, and New York State Psychiatric Institute. The Committee meets on a quarterly basis. The Radiation Safety Officer reports on professional and on technical matters to Dr. Hall, chair of the JRSC and reports budgetary matters to Dr. Lewy, who represents Dr. Gerald D. Fischbach, the Dean of CUMC. The Radiation Safety Office participates in the review of research protocols for the Radioactive Drug Research Committee under the jurisdiction of the U.S. Food and Drug Administration.

Radiation Safety Office staff are Columbia University Medical Center employees. Columbia University Medical Center (CUMC), New York Presbyterian Hospital (NYPH), and the New York State Psychiatric Institute (NYSPI) fund the Radiation Safety Office budget via a cost sharing payback arrangement.

OVERVIEW OF RADIATION SAFETY OFFICE RESPONSIBILITIES

Collectively, Columbia University Medical Center, New York Presbyterian Hospital and New York State Psychiatric Institute form a large health sciences complex with extensive teaching, research, and clinical facilities. The basic goal of the Radiation Safety Office is to ensure the implementation of all protective measures necessary to ensure that the dose from ionizing radiation to patients, visitors, students, faculty and staff on campus, as well as to the general community at large, is As Low As Reasonably Achievable (ALARA). Major entities supported by the Radiation Safety Office are:

- Columbia University Medical Center
- New York Presbyterian Hospital
- New York State Psychiatric Institute
- Columbia Presbyterian Eastside
- New York Presbyterian Hospital, Allen Pavilion
- Columbia University Medical Center Cyclotron Facility
  Operated by PETNet Pharmaceuticals Inc.
- Dental Facilities throughout CUMC and elsewhere as described later in this report.

Ongoing construction and development has recently added the following buildings to the Columbia University Medical Center: the Russ Berrie Pavilion, the Mary Woodard Lasker Building, and the Irving Cancer Research Center. The projected completion of several additional buildings will add to the responsibilities of the Radiation Safety Office in the near future.

Reporting to the Joint Radiation Safety Committee of Columbia University Medical Center, New York Presbyterian Hospital, and the New York State Psychiatric Institute, the Radiation Safety Officer and the staff of the Radiation Safety Office are responsible for obtaining and maintaining licenses authorizing the possession and use of radioactive materials and obtaining and maintaining registrations and permits for the operation of radiation producing equipment. In addition, the Radiation Safety Office is responsible for obtaining and maintaining those permits necessary for the safe disposal or controlled release of research and medical wastes containing radioactivity.

The Radiation Safety Office ensures compliance of authorized users of radioactive materials or radiation producing equipment with all governmental regulatory requirements.
and guidelines by means of: training, education, consultation, and by a program of internal audits and inspections of facilities. Regulatory agencies charged with overseeing the possession, use, or disposal of radioactive materials or radiation producing machines are:

- United States Food and Drug Administration
- United States Nuclear Regulatory Commission
- United States Environmental Protection Agency
- New York State Department of Environmental Conservation
- New York State Department of Health
- New York City Department of Health and Mental Hygiene, Office of Radiological Health
- New York City Health Department, the City of New York Radioactive Materials Licenses, Registrations and Permits in- 

The New York City Department of Health, the New York State Department of Environmental Conservation, and the United States Food and Drug Administration conduct periodic inspections and audits of the Columbia University Medical Center, New York State Psychiatric Institute, and New York Presbyterian Hospital facilities operating under their licenses or permits. The Radiation Safety Office works continuously to ensure that regulatory violations are prevented and to ensure those that do occur are swiftly corrected.

The Radiation Safety Office also ensures compliance with institutional policies and procedures published in the “Radiation Code and Guide of Columbia Presbyterian Medical Center and New York State Psychiatric Institute.”

**SUMMARY OF RADIATION SAFETY OFFICE OPERATIONS FOR 2004**

A summary of activities performed and services provided by the Radiation Safety Office is presented below. While inclusive of most major activities and services, the summary is by no means exhaustive, but is intended to provide a representative overview of departmental operations. An unabridged compilation of Radiation Safety Office activities and services may be found in the Minutes of the Quarterly Meetings of the Joint Radiation Safety Committee of Columbia University Medical Center, New York Presbyterian Hospital, and the New York State Psychiatric Institute.

Statistical data presented are from the fiscal year, July 1, 2003 through June 30, 2004. Activities are covered up to February 11, 2005.

**Maintenance of New York City Department of Health and Mental Hygiene, Office of Radiological Health Licenses, Registrations, and Permits, Audits and Inspections**

A primary activity of the Radiation Safety Office is the continued maintenance of the City of New York Radioactive Materials Licenses, the Certified Linac Registration, and the X-Ray Permits. Currently this includes:

- Radioactive Materials License No. 75-2878-01 (Broad Scope Human Use)
- Radioactive Materials License No. 92-2878-02 (Teletherapy)
- Radioactive Materials License No. 74-2878-03 (Non-Human Use)
- Radioactive License No. 52-2878-04 (Cyclotron Facility)
- Radioactive Materials License No. 93-2878-05 (Gamma Knife)
- City of New York Therapeutic Radiation LINAC Unit Certified Registration No. 77-0000019.
- Columbia-Presbyterian Hospital Radiation Installation Permit H96 0076353 86
- Columbia-Presbyterian-Allen Pavilion Radiation Installation Permit H96 0076383 86

Significant activities performed in 2004 to maintain the City of New York Licenses, Registrations and Permits include:

1. On March 30, 2004, the Radiation Safety Office received a request from PETNET Pharmaceuticals Inc., to amend License 52-2878-04 to add Beth Kraemer as an Authorized Nuclear Pharmacist, John Cisneros and Kurt Lagenwalter as authorized cyclotron operators. On July 19, 2004, a further request was made to add Chris Grosch as an authorized cyclotron operator/engineer. Following the receipt and review of relevant credentials and documentation, review and approval of the JRSC executive committee, the Radiation Safety Office forwarded these amendment requests to the NYC Dept. of Health, Office of Radiological Health. These actions were approved by a quorum of the JRSC during the quarterly meeting held on September 13, 2004.

2. On March 3, 2004, the Radiation Safety Office received a request from Ronald Van Heertum, M.D., Vice-Chairman, Radiology, to amend the Broad Scope Human Use Radioactive Materials License 75-2878-01 to allow for the installation and operation of a Siemens Biograph PET/CT scanner to be housed in a Medical Coaches Mobile Scanner Trailer at a fixed location at the Columbia University Medical Center. This request was approved by a quorum of the Joint Radiation Safety Committee during their quarterly meeting on March 23, 2004.

During April 2004, the Radiation Safety Office completed a radiation safety survey and evaluation of the newly installed PET/CT trailer. A written report of the survey was submitted to Dr. Ronald Van Heertum and Dr. Peter Esser.

During June 2004, the PET Suite’s administration requested that the Radiation Safety Office outline the requirements for the commencement of human subject research in the PET/CT Trailer. The Radiation Safety Office responded to this request by submitting a document titled Draft Radiation Safety Requirements for PET/CT Trailer to the PET Subcommittee. This document references the applicable License conditions, regulatory requirements, and regulatory guidelines to be followed in the performance of routine diagnostic and human subject research in the PET/CT Trailer. A draft summary of recommended policies and procedures was also presented.

3. On March 11, 2004 the Radiation Safety Office responded to a request to provide the New York City Department of Health and Mental Hygiene, Office of Radiological Health with an inventory of radioisotopes with half-lives greater than 120 days currently in our possession. The purpose of this request was to provide information necessary to determine the University’s decommissioning filing status, as
required by Article 175 section 101(n).

On April 20, 2004, the Radiation Safety Office received notification that CUMC, NYPH and Columbia University Morningside were liable for financial reassignment for de-commissioning in the prescribed amount of $825,000.

In May 2004, Mr. Hatami, Assistant Director of the Radiation Safety Office, Ms. Mah, Director of Risk Management for Columbia University, and Dr. Hayes, Radioactive Materials Division of the Office of Radiological Health, completed negotiations to ensure that both the Medical Center and Morningside campus were covered to the required extent.

4. On July 31, 2004, the Radiation Safety Office submitted to the New York City Department of Health Office of Radiological Health an application for renewal of the Teletherapy License (92-2878-02) for an additional five (5) year period.

5. During 2004, the Radiation Safety Office reminded the Joint Radiation Safety Committee and all Authorized Users of several procedural and administrative requirements. The specifics of these reminders may be found in the quarterly reports of the Radiation Safety Office. Briefly, some of the topics covered were:

- Authorized Users need to report to the Radiation Safety Office any changes in the following: (1) handling or experimental procedures related to the use of radionuclides; (2) quantities and chemical/physical forms of radionuclides used; or (3) therapy physicists, authorized technicians, and radiation safety managers who use or oversee the use of radioactive materials.
- Human-use research protocols require IRB review at least once a year.
- The U.S. Food and Drug Administration recommends, as good practice, that all DMFs be updated annually.
- Medical Physicists practicing in New York State are required to obtain professional licensure from the New York State Department of Education. At present, all senior officers of the Radiation Safety Office are certified either by the American Board of Health Physics, the American Board of Medical Physics, and/or the American Board of Radiology and are licensed to practice as Medical Physicists by the State of New York.
- RCNY 175.103(2) requires that: “(ii) The radiation safety officer shall: (A) investigate overexposures, misadministrations, accidents, spills, losses, thefts, unauthorized receipts, uses, transfers, and disposals, and other deviations from approved radiation safety practice and implement corrective actions as necessary.”

6. The New York City Department of Health, Office of Radiological Health conducts periodic audits of records and inspections of facilities at the Columbia University Medical Center operating under the Radioactive Material Licenses, the Certified Linac Registration, and the Diagnostic X-ray Registrations. In 2004, these audits and inspections included:

- April 7, 2004 License 93-2878-05 (Gamma Knife)
- March 10, 2004 License 75-2878-01 (Human Use License of Broad Scope)

7. At the Joint Radiation Safety Committee Meetings of March 10, 2004 and June 1, 2004, extensive discussion was held regarding the advantages and disadvantages of combining the Office of Environmental Health and Safety with the Radiation Safety Office and forming a single Office of Radiation Safety and Environmental Health. Robert Lewy, M.D., Senior Associate Dean, Faculty Medicine/Administration, attended the meetings and pointed out to the Committee some of the possible efficiencies in operation that might result from such a merger. Richard Borri, Senior Scientist, New York City Department of Health and Mental Hygiene (NYCDOH&MH) also attended the JRSC meeting of March 10, 2004. In a Notice addressed to Columbia University Medical Center dated August 2, 2004, Mr. Borri informed CUMC that such a merger could not take place without prior approval of both the JRSC and the NYCDOH&MH. A subsidiary issue was also raised concerning the requirement for professional licensure by the New York State Department of Education of all individuals practicing health physics at a medical institution.

In addition, unlike the EH&S, the Radiation Safety Office is responsible for providing radiation safety and medical health physics services to three institutions, CUMC, NYPH, and NYSPI. Any proposed changes to the organizational structure would require review and approval of all three institutions.

In a reply to the NYCDOH&MH August 23, 2004, Gerald D. Fischbach, M.D., Dean, Faculty of Medicine, CUMC, informed Mr. Borri and the NYCDOH&MH that such a merger would not take place; rather the independence of the Radiation Safety Office was stressed to insure that the RSO has sufficient authority and organizational freedom to identify and correct radiation safety problems.

8. At the Joint Radiation Safety Committee Meetings of March 10, 2004 Richard Sohn, Ph.D., the CUMC management representative to the Joint Radiation Safety Committee notified the Committee that he had accepted a position with Case Western University in Cleveland, Ohio. On June 1, 2004, the Joint Radiation Safety Committee voted to replace Dr. Sohn, as the management representative to the JRSC with Robert Lewy, M.D., Senior Associate Dean, Faculty Medicine/Administration. Dr. Lewy is responsible for budgetary and financial oversight of the Radiation Safety Office.

9. During 2004, the Radiation Safety Office suspended two individuals from work following reports that their personnel monitoring badges had received doses in excess of their annual limits. These incidents, described below, were investigated extensively and reported to the New York City Department of Health. More detailed descriptions of these events may be found in the Quarterly ALARA reports of the Radiation Safety Office.

On April 19, 2004, Landauer Inc. notified the Radiation Safety Office that the annual effective dose equivalent for participant 89839 was 7602 mrem, which exceeds the annul whole body exposure limit of 5000 mrem. This individual was immediately suspended from working with radiation, and Richard Borri at the NYCDOH was informed of the situation. The follow-up inspection revealed that this individual had misused badges. Based on the results of this in-
spection, the individual’s effective dose equivalent is believed to be closer to 2221 mrem. A review of the results of the follow-up inspection was submitted to the NYCDOH.

On November 12, 2004, Landauer Inc. notified the Radiation Safety Office that the annual effective dose equivalent for participant 89571 was 9513 mrem, which exceeds the annual whole body exposure limit of 5000 mrem. The follow-up inspection revealed that this individual had stored badges within the procedure room. Based on this finding and the individual’s previous exposure history, the Radiation Safety Office requested that this badge reading be removed. A summary of the results of the follow-up inspection was submitted to the NYCDOH.

10. On September 3, 2004, David Strauss, M.D., Chairman, New York State Psychiatric Institute Institutional Review Board and Eric Hall, D.Phil., Chairman, Joint Radiation Safety Committee were notified that quality control tests for two (2) human subject research studies performed on August 30, 2004 and September 1, 2004 indicated that the radio-labeled compound administered to the subjects may not have been the compound designated in the research protocols. Dr. Strauss and Dr. Hall immediately required the researcher to suspend human subject research protocols using the compound in question.

On September 13, 2004, at a meeting of the Joint Radiation Safety Committee, this incident was reported to the JRSC by the Chairman of the Drug-Master File Subcommittee, T. Lee Collier, Ph.D. At that meeting, the JRSC voted to suspend all human use research protocols of the Responsible Investigator involved.

The Radiation Safety Office investigation of this incident was performed primarily by Thomas W. Juchnewicz, M.S., D.A.B.R., Assistant Radiation Safety Officer. Draft reports of the on-going investigation were presented by the Radiation Safety Office to the Joint Radiation Committee at emergency meetings on September 29 and November 5, 2004. At the September 29, 2004 meeting, the JRSC voted to revoke the researcher’s privileges as a Responsible Investigator for human subject research. At the November 5, 2004 meeting, the JRSC reviewed the action they had taken on September 29, 2004 and voted to confirm it.

The final draft report of the Radiation Safety Office to the JRSC, dated November 5, 2004, concluded that the incident resulted in two diagnostic misadministrations that were not reportable to the NYCDOH/RMH. The Radiation Safety Office recommendations for corrective actions by the Department in question, the Department of Functional Brain Imaging, were submitted for the review and approval of the JRSC.

In addition to the two diagnostic misadministrations, the Radiation Safety Office informed the JRSC that the incidents of August 30, 2004 and September 1, 2004 appeared to constitute an “unexpected adverse drug experience” under 21 CFR 312.32. This is the lowest form of error in a clinical research protocol. As required by regulation, the sponsor for the human subject research protocols informed the U.S. Food and Drug Administration of the incident. The U.S.F.D.A. District Office suspended all human subject research using any radio-labeled compounds manufactured in the radioisotope laboratory in which the suspect compound originated pending the results of an audit and inspection. The U.S.F.D.A. District Office conducted the audit and inspection of the laboratory from October 25 through November 10, 2004. On November 10, 2004, the U.S.F.D.A. issued a list of specific corrective actions with regard to this laboratory. At present these corrective actions are being implemented under the review of the Chairman of the JRSC.

11. In 2004, major steps were taken in the transfer of the responsibilities for radiation safety in the Cyclotron facility from the Radiation Safety Office to PETNet Pharmaceuticals Inc. These responsibilities are being transferred in a multi-step process that originated in a decision made by Harvey Colten, M.D., Vice President, Health Science Division, on December 9, 2003.

During 2004, PETNet submitted a Standard Operating Procedure Manual to the Radiation Safety Office for review and is training employees in the proper methods of carrying out all required radiation safety responsibilities. Radiation safety responsibilities are being performed independently by both the Radiation Safety Office and PETNet Pharmaceuticals Inc. during this training period. This allows for evaluation of PETNet’s performance relative to the performance of the Radiation Safety Office. During this training period, the Radiation Safety Office is ceasing to perform any tasks that PETNet Pharmaceuticals Inc. demonstrates itself capable of suitably carrying out without Radiation Safety Office support. The Radiation Safety Office will function strictly as an oversight body once the PETNet’s Standard Operating Procedure manual is fully approved and all radiation safety responsibilities have been transferred. This role will be similar to the Radiation Safety Office’s role in other clinical departments throughout CUMC, NYPH, and NYSPI.

The transfer of responsibilities from the Radiation Safety Office to PETNet Pharmaceuticals Inc. was formally declared on March 25, 2004, when the Radiation Safety Office submitted a request to the NYCDOH to remove the Memorandum of Understanding dated November 22, 1994 from the CUMC license renewal application (dated September 29, 2002). As required, submission of this license amendment request occurred following approval by the JRSC.

**Maintenance of New York State Department of Environmental Conservation Permits, Audits and Inspections**

Another primary activity of the Radiation Safety Office is the continued maintenance of New York State Department of Environmental Conservation Radiation Control Permit No. 2-6201-00005/00006.

Under the Conditions of the Radiation Control Permit and in compliance with New York State 6 NYCRR Part 380, Rules and Regulations for Prevention and Control of Environmental Pollution by Radioactive Materials, Columbia University Medical Center conducts medical research and clinical activities that discharge limited and controlled quantities of radioisotopes to the atmosphere and to sewage systems.

Columbia University Medical Center is sited within a densely populated urban area. The quantities of radioisotopes discharged and the resulting public radiation dose are
closely regulated by the New York State Department of Environmental Conservation. Radiation doses to the general public resulting from atmospheric discharges of radioisotopes are required not to exceed the USNRC Constraint Limit of 10 mrem per year.

Columbia University Medical Center and the New York State Psychiatric Institute are currently permitted a total of fifteen (15) atmospheric emission points from which radionuclides are discharged to the atmosphere. Monitoring, analyzing, reporting, and minimizing discharges from these emission points, in order to ensure compliance with the Conditions of the Radiation Control Permit, is one of the major continuing activities of the Radiation Safety Office.

Significant activities performed in 2004 to maintain the New York State Department of Environmental Conservation Radiation Control Permit include:

1. As required by New York State 6 NYCRR Part 380 and the Conditions of the New York State Department of Environmental Conservation Radiation Control Permit, the Radiation Safety Office will be submitting an Annual Report summarizing Discharges of Radioactive Effluents to the Environment from the fifteen atmospheric emission points and by controlled sewer disposal by the end of March 2005. For the calendar year 2004, all atmospheric discharges were within the quantities authorized by the Radiation Control Permit, and the resulting public dose was within the U.S.N.R.C. constraint limit of 10 millirems per year. All discharges to sewers were well below the Effluent Concentration Limits as required by 6 NYCRR Part 380-11.7, Table of Concentrations.

2. On March 10 and 11, 2004, the New York State Department of Environmental Conservation conducted an unannounced audit of records and an inspection of facilities at the Columbia University Medical Center, New York Presbyterian Hospital, and the New York State Psychiatric Institute under NYSDEC Radiation Control Permit 2-6201-0005/0006. On April 5, 2004, the Radiation Safety Office received a letter from Markus Spivak of the NYSDEC that summarized the results and recommendations of the inspection. This letter stated that, “Within the scope of the inspection, operations at CPMC were found to be in compliance with Part 380 and the conditions of its permit.” However, the letter recommended that the following items be incorporated into the Radiation Safety Office program: (1) staff members intimately involved in overseeing cyclotron operations should undergo additional training; (2) the continuous flow rate monitor in the cyclotron stack should be replaced; and (3) a rooftop filter should be installed for the Nuclear Medicine exhaust stack in order to reduce the emission of I-131.

The Radiation Safety Office responded to these recommendations in the following ways: (1) Salmen Loksen, Director of the Radiation Safety Office, and Thomas Juchniewicz, Assistant Radiation Safety Officer, attended a training program sponsored by the US Particle Accelerator School at the University of California, Berkeley, from January 7, 2005 to January 21, 2005. (2) Thermoelectron Corporation, the original installers of the continuous flow rate monitor in the Radioligand Laboratory exhaust stack, have installed a similar continuous flow rate monitor in the Cyclotron exhaust stack. (3) During September 2004, Taggart Associates installed a filter on the Nuclear Medicine exhaust stack.

3. As required by the Conditions of NYSDEC Radiation Control Permit 2-6201-0005/0006, the flow of all exhaust stacks authorized to release controlled amounts of radionuclides to the atmosphere was independently measured by International Testing and Balancing Ltd. on June 9 and 10, 2004. The results of these measurements were reported to the Radiation Safety Office on June 24, 2004.

4. On May 8, 2004, as required by the Conditions of NYSDEC Radiation Control Permit 2-6201-0005/0006, all filters in the Cyclotron, Radioligand, and PET Suite stack monitoring systems were replaced. The Radiation Safety Office recommended that several changes be made to the PETNet Pharmaceutical’s Standard Operating Procedures Manual. These recommendations are detailed in the Quarterly Report of the Radiation Safety Office dated December 15, 2004.

5. As required by 6 NYCRR Part 380 and the conditions of our NYSDEC Radiation Control Permit, the Radiation Safety Office has reviewed atmospheric discharges of volatile isotopes of Iodine and Xenon-133 gas. From January 1 through August 31, 2004, the amount of Iodine-131 discharged from Emission Point 1, the Nuclear Medicine Stack located on the rooftop of the Milstein Hospital Building, was 12% of the permit limit. From January 1 through June 30, 2004, the amount of Xenon-133 discharged from Emission Point 1 was 3% of the permit limit. These trends indicate that the 2004 total annual discharge will be well below the maximum quantity authorized by our permit.

6. On May 8, 2004, as required by 6 NYCRR Part 380 and the conditions of our NYSDEC Radiation Control Permit, the Radiation Safety Office has reviewed atmospheric discharges of volatile isotopes of Iodine and Xenon-133 gas. On May 8, 2004, as required by the Conditions of NYSDEC Radiation Control Permit 2-6201-0005/0006, all filters in the Cyclotron, Radioligand, and PET Suite stack monitoring systems were replaced.

7. The Radiation Safety Office reminded all Authorized Users whose laboratories discharge radioisotopes to the atmosphere that, in accordance with New York State Department of Environmental Conservation, Application Guidelines For Radiation Control Permits For Discharge Of Radioactive Materials In Effluents To Air, E. Permit Modifications, Columbia University Medical Center is required to conduct its Radiation Safety Program in accordance with the statements, representations, and procedures contained in the application and supporting documents of NYSDEC Radiation Control Permit 2-6201-0005/0006. The Guidelines require that, before any changes are made to operations, facilities, equipment, procedures, or radioactive materials that affect discharges, the proposed changes must be submitted to the Joint Radiation Safety Committee for review and approval prior to being submitted to the New York State Department of Environmental Conservation as a Permit Amendment Request.

Administration of Radioactive Material: Receipt, Distribution, and Radioactive Waste Disposal

A major program of the Radiation Safety Office is the centralized administration of all authorized radioactive materials used at the Columbia University Medical Center, New
York Presbyterian Hospital, and the New York State Psychiatric Institute.

Types of radioisotopes, allowed uses, and possession limits for member Institutions and major Departments are authorized under five separate City of New York Radioactive Materials Licenses.

The use of authorized radioisotopes by individual Authorized Users and Responsible Investigators is controlled by the Joint Radiation Safety Committee through the administration of the Radiation Safety Office. Human Use of radioactive materials by Authorized User Physicians is allowed after a review of credentials and a majority vote by a quorum of the Joint Radiation Safety Committee. Non-Human Use of radioactive materials is granted after a review of applications and written permission of the Radiation Safety Officer. In 2004, 12 new Responsible Investigators were reviewed and approved for non-human use of radioactive materials, and 51 current Responsible Investigators received renewal of their authorizations.

Significant activities performed in 2004 to administer, receive, distribute, and dispose of radioactive materials included:

1. In October 2004, in accordance with the current EPA Audit Policy: “Incentives for Self-Policing: Discovery, Disclosure, Correction and Prevention of Violations,” CUMC began an environmental self-audit program. The self-audit program was performed in partnership with Woodard and Curran, an environmental consulting firm. The goal of the self-audit program was to internally identify and correct violations of EPA regulations, thereby minimizing or avoiding any fiscal penalties that might have been imposed had the violations been identified by the EPA itself. Most of the Radiation Safety Office’s activities are related to the use of non-hazardous, byproduct material and are thus regulated by the New York City Department of Health under agreement with the Nuclear Regulatory Commission. Such activities lie outside the regulatory scope of the EPA and of the self-audit. However, the audit did bring the current CUMC Mixed Waste policy under scrutiny. This is because Mixed Waste, as defined by the EPA, is “waste that contains a hazardous waste component and a radioactive component,” and is thus regulated by both the EPA and the Nuclear Regulatory Commission. The following gives a partial list of actions taken and changes that were introduced into the Radiation Safety Office’s procedures following the self-audit: recycling/redistribution procedures for lead shielding and uranyl compounds were formalized; the procedures that each Responsible Investigator (RI) uses to generate and store mixed waste were being carefully reviewed and audited when the RI applies for renewal of a radioactive materials license; the frequency of Environmental Health and Safety/Radiation Safety cross training sessions was increased; a more elaborate waste tracking system was introduced; and training was provided to lab managers whose lab’s generate mixed waste.

2. The Radiation Safety Office received and distributed 2791 packages containing radioactive material, excluding shipments to the Nuclear Medicine and Radiation Oncology departments. The Radiation Safety Office had issued prior approval for all received shipments. Package surveys and wipe tests were also conducted to ensure that none of the packages were contaminated. The Radiation Safety Office maintains inventory control of all radioactive materials received and distributed through the use of a detailed and extensive computerized database.

3. On February 26, 2004, the Radiation Safety Office shipped seventeen 30-gallon drums of liquid scintillation vials (LSV) for disposal by NSSI Source & Services of Texas via RADIAC Research Corp. The total volume of the LSV shipment was 68.2 cubic feet, weighing 2,550 pounds. The total activity shipped was 5.548 mCi, of which 3.59 mCi was tritium (H-3), 1.638 mCi was C-14, and 0.32 mCi was other isotopes.

4. On August 19, 2004, the Radiation Safety Office shipped 8.4 gallon of low-level mixed waste via Radiac Research Corp. The waste contained 5.1 mCi of H-3, and 2.2 mCi of S-35. The waste also contained chloroform, methanol, heptane, and other hazardous chemicals.

5. On October 28, 2004, the Radiation Safety Office collected all hazardous materials found in its storage facilities and from research labs and arranged a shipment via Radiac Research Corp. Included in the shipment were liquid scintillation vials, liquid mixed waste, radioactive CO gas, uranyl, and thorium compounds. The waste contained totally 28.306 mCi of radioactive materials, including 19.387 mCi of H-3, 5.139 mCi of C-14, 3.66 mCi of S-35, and 0.12 mCi of other radionuclides.

6. On December 11, 2004 the Radiation Safety Office shipped a total of fifty-seven drums of Dry Waste (six 55-gallon and fifty-one 30-gallon drums) for disposal by Envirocare of Utah via GTS Duratek Super-Compaction. On the same day, the Radiation Safety Office shipped one 55-gallon and one 30-gallon fiber drums of solid biological waste, and four 14-gallon fiber drums of solid animal carcasses for incineration at Envirocare of Utah. The total volume of the dry waste shipment was 249.51 cubic feet, weighing 4,925 pounds. The total dry waste activity shipped was 117.68 mCi, of which 106.97 mCi was tritium (H-3), 6.02 mCi was C-14, and 4.69 mCi was S-35. The total volume of biological waste shipped was 11.51 cubic feet, weighing 135 pounds, containing 2.9 mCi of tritium and 0.31 mCi of other radionuclides. The total volume of animal waste shipped was 7.44 cubic feet, weighing 107 pounds, containing 25.6 mCi of tritium (H-3) and 4.82 mCi of other radionuclides. The pickup was made from the P&S Building storage facility during 3 a.m. and 5 a.m., and the assistance of the Security staff was very helpful in this procedure.

7. On October 30, 2004, the Radiation Safety Office shipped Dry Active Waste for disposal via Duratek, Inc. The shipment included seventeen 30-gallon metal drums of dry solid waste, ten 55-gallon metal drum of dry solid waste, and one 14-gallon fiber drum of animal waste. The waste contained 104.21 mCi of various radionuclides, including 89.3 mCi of H-3, 8.97 mCi of C-14, 0.25 mCi of P-32, 3.75 mCi of S-35, 1.26 mCi of Ca-45, and 0.68 mCi of Fe-55.

8. As of June 30, 2004, the Radiation Safety Office had disposed of approximately 3765 liters of low-level aqueous radioactive waste through monitored sewer dis-
9. As of June 30, 2004, the Radiation Safety Office had disposed of approximately 3000 gallons of fully decayed short half-life radioactive waste that had been stored for more than ten half-lives. All containers were surveyed and analyzed to ensure that there was no detectable radiation prior to disposal of this waste as ordinary waste.

10. As required by 6 NYCRR Part 380 and the conditions of our NYSDEC Radiation Control Permit, the Radiation Safety Office reviewed controlled sewer disposal of aqueous radionuclides. During the entire fiscal year, the discharge for all isotopes was well below the concentration limits of 6NYCRR Part 380-11.7 Table II.

11. As of June 30, 2004, the Radiation Safety Office had picked up 944 waste containers from research labs and delivered 950 waste containers. During the same period, the Radiation Safety Office picked up 250 bags of patient waste from clinical areas.

12. During the fiscal year of 2004 the Radiation Safety Office approved 1126 purchase orders for materials that contain radioisotopes. The above orders resulted in the purchase of a total of approximately 31 Curies of activity. $^{35}$S, $^{3}$H, $^{32}$P, $^{103}$Pd (seeds), and $^{125}$I, $^{192}$Ir (seeds), and $^{133}$Xe (gas) were the isotopes purchased with the highest activities.

13. On January 26, 2004, the Radiation Safety Office and Environmental Health & Safety (EH&S) staff jointly issued the official CUMC mixed waste policy. Supported by the audit section of the Radiation Safety Office, waste area staff has started monitoring liquid waste that might contain hazardous chemicals. As of this quarter, the Radiation Safety Office has identified approximately 20 labs that have produced or are currently producing mixed waste. These labs are properly minimizing the amount of mixed waste produced. As of this quarter, there is no known hazardous or mixed waste in the Radiation Safety Office storage facilities.

14. During October 2004, the Radiation Safety Office distributed two memoranda to all responsible investigators. The first memorandum, dated October 10, 2004 and addressed from Mr. Hatami, stated that the Radiation Safety Office was collecting unwanted/orphaned sealed sources, uranyl acetate, scintillation fluid, and equipment. This collection initiative was part of a campus-wide clean up project that is being funded by the central administration of Columbia University. The sealed sources collected during this clean up initiative will be disposed of by one of several companies from which Mr. Hatami is currently receiving bids. The cost of this shipment is expected to be approximately $130,000. The second memorandum, dated October 27, 2004 and addressed from Mr. Loksen, stated that the Radiation Safety Office maintains a stock of surplus materials and supplies that are available for redistribution to interested labs. These materials include lead bricks and pigs, check sources, and plastic shielding.

15. On July 6, 2004 the Radiation Safety Office shipped sealed sources containing 801 GBq (or 21.6 Ci) of Am 241 to NSSI for recovery. The sources were previously sealed into a capsule by Los Alamos National Laboratory staff and were shipped as a special form in a stainless steel capsule. The packaging of these sources, a 5-gallon metal drum, was picked up by Federal Express on July 26 and was received by NSSI on July 27.

16. On February 6, 2004, three Pu/Be sources with a total weight of 30.13 grams were shipped to Los Alamos National Laboratory (LANL) as per the instructions given by the Off-Site Source Recovery Project of LANL. Packaging was performed by CoPhysics.

17. A meeting was held to discuss problems associated with the pickup of Cyclotron doses from the NYPH to be delivered to other hospitals. Attending the meeting were Salmen Loksen and Dr. Jacob Kamen from the Radiation Safety Office, Tigran Sinanian of PETNET Pharmaceuticals, and Richard Irizarry, head of NYPH Security. On May 7, 2004, Medical Delivery Services, Inc., a company contracted by PETNET to pick up and deliver pharmaceuticals, agreed to have a maximum of three drivers at a time in the hospital parking area. Each will display signage and limit their pickup times to 45 minutes. This agreement was acceptable to NYPH security, which will monitor for compliance.

**ALARA Program – Personnel Dosimetry, Bioassay, and Area Monitoring**

In accordance with regulatory requirements, the Radiation Safety Office operates an ALARA (As Low As Reasonably Achievable) Program to ensure that the radiation doses to all workers at the Columbia University Medical Center, New York Presbyterian Hospital, and New York State Psychiatric Institute and the radiation doses to the general public resulting from all operations of Columbia University Medical Center, New York Presbyterian Hospital, and the New York Psychiatric Institute are within the legal limits and As Low As Reasonably Achievable (ALARA).

The principal methods of monitoring radiation dose are the assignment of personnel radiation dosimeters to individuals, the posting of area and environmental dosimeters, and the monitoring of all discharges containing radioactivity.

Immediate action is taken, as appropriate, in response to unusual or high dosimeter readings. Quarterly ALARA Reports are prepared and submitted to the Joint Radiation Safety Committee. The Quarterly ALARA Report presents: the doses of individual workers that exceed ALARA I Limits; the results of investigation of doses to individual workers that exceed ALARA II Limits; and discussions of significant trends within departments that may experience high individual doses. In addition, the Quarterly Environmental ALARA Report is prepared and submitted to the Joint Radiation Safety Committee. The Quarterly Environmental ALARA Report presents the quantities of radionuclides discharged to the atmosphere and the sewer system and the resulting dose to the general public.

As discussed previously in this report, in 2004, all doses to individual workers were less than the legal annual reportable limits as specified in RCNY Article 175, Radiation Control, except for one person from the Angiography lab who misused his badges and one person from the Cardiovascular lab who stored her badge in the procedure room. All doses to the general public resulting from atmospheric dis-
charges of radionuclides were less than the U.S.N.R.C. constraint limit of 10 mrem per year.

Significant activities performed in 2004 to maintain the ALARA Program were:

1. The Radiation Safety Office distributed approximately 9,000 personnel radiation dosimeters each quarter, including both monthly and quarterly badges. A total of approximately 38,000 dosimeters were distributed and collected in 2004. To maintain dosimetry records, the Radiation Safety Office uses dedicated computers with Internet and direct modem access to the database of the dosimeter supplier, Landauer Inc.

2. The Radiation Safety Office received Annual Occupational Exposure Reports (NRC Form 5) from Landauer Inc. for the year 2003 and forwarded these reports to radiation workers as required by the New York City Department of Health regulations.

3. In 2004, the Radiation Safety Office notified 41 employees with ALARA Level 1 readings and investigated 14 cases of ALARA Level II readings as reported by Landauer Inc. Particular attention was paid to occupational groups that typically exceed the ALARA limits, i.e., workers and researchers at the Cyclotron Facility, Angiography, the Cardiac Cath Lab, and physicians in the PET Suite.

4. In 2004, the Radiation Safety Office performed 33 thyroid bioassays on radiation workers using radioactive isotopes of iodine including Iodine-123, Iodine-125, and Iodine-131.

5. The Radiation Safety Office provided all workers who had declared pregnancy with health physics counseling about risk factors. Also, additional monitoring of the fetus during the gestation period was provided, and personnel radiation exposure reports were closely followed. The work environments were evaluated and modified if necessary.

Radiation Safety Compliance – Routine Internal Inspections and Audits

A major activity of the Radiation Safety Office is the performance of facilities inspections and audits of records of approved clinical departments and research laboratories to ensure compliance with regulatory requirements as well as guidelines and policies of the Joint Radiation Safety Committee.

Significant compliance activities conducted in 2004 include:

1. The Radiation Safety Office completed annual inspections and audits of CUMC, New York Presbyterian Hospital, and New York State Psychiatric Institute clinical facilities using radioactive materials. The audits and inspections are to ensure compliance with City of New York Radioactive Materials License conditions and with RCNY Article 175, Radiation Control. The facilities audited include: New York Presbyterian Hospital Nuclear Cardiology, NYP Nuclear Medicine, Allen Pavilion Nuclear Cardiology, and Allen Nuclear Medicine. In addition, the Radiation Safety Office performed the required quarterly inventory and leak testing for all radioactive sources located in the following facilities: Milstein Nuclear Medicine, Allen Pavilion Nuclear Medicine, Cyclotron, and Columbia University Health Sciences (VC-11 Alpha sources, Irradiators, etc.). All were found to be in compliance. Leak Test Certificates were generated and issued for each of the above sealed sources.

2. In 2004, the Radiation Safety Office performed 1159 routine radiation safety inspections and audits of Columbia University Medical Center and New York State Psychiatric Institute research laboratories using radioactive materials. The results were communicated to the Responsible Investigators. A total of 190 deficiencies were followed up by correction of the cited deficiencies. During the same period, the Radiation Safety Office conducted 100 laboratory clearance and exit and entry surveys.

3. In 2004, the Radiation Safety Office measured airflow rates in 84 fume hoods in areas where volatile radioactive materials are used. In all rooms where radioactive gases or aerosols are used, ventilation rates were measured, and Spill Gas Clearance Times were calculated and posted. Adjustments were made as required to air supply and exhaust systems to obtain negative pressure conditions. Researchers whose hoods did not meet safe flow rate standards were instructed to have their hoods repaired or replaced. There are 34 fume hoods for I-125 or other volatile isotopes.

4. In addition to the regular inspections of clinical facilities and research laboratories, the Radiation Safety Office investigates major spills, incidents involving radioactive materials, and misadministrations. The Radiation Safety Office ensures that, when required, timely notice of reportable incidents is made to the New York City Department of Health, Office of Radiological Health. The Radiation Safety Office responded to the following 7 spill incidents in 2004. Further details are on file in the Radiation Safety Office and may also be found in the Radiation Safety Office’s Quarterly reports to the JRSC.

- On March 26, 2004, a Tc-99m spill occurred in the Nuclear Cardiology Department. After proper decontamination procedures, residual surface contamination was well below the levels specified in Article 175.

- On March 30, 2004, a C-11 spill occurred in the Pet Suite located in Milstein Hospital Basement Room 3-128. The area of the spill was cleaned and avoided for 3 hours (10 half-lives) following the spill.

- On April 23, 2004, Tl-201 spill occurred in the Nuclear Cardiology Department during a stress test. Any contaminated equipment was placed in storage for one month (10 half-lives). The room was wipe tested and ambient dose rates were measured and determined to be below the levels specified in Article 175 before normal activity resumed within the room.

- On July 21, 2004, a Tc-99m spill occurred in the Nuclear Cardiology Department. After proper decontamination procedures, residual surface contamination was well within the limits of Article 175.

- On August 3, 2004, a worker reported that he believed himself to be contaminated because a Geiger meter had measured high readings on his hands after he had worked with 32P. A physicist from the Radiation Safety Office surveyed and conducted an interview with this individual and determined that contamination had not occurred, but rather that the individual had used the Geiger meter too
close to a radiation source.

- On December 3, 2004, a Tc-99m spill occurred in Room 3-116 of the Allen Pavilion. Contaminated areas were covered with lead while the isotope decayed.
- On December 21, 2004, a Co-57/I-125 spill occurred in Room 155 of the Psychiatric Annex Building. After proper decontamination procedures, residual surface contamination was well within the limits of Article 175.

5. On August 31, 2004, the CUMC Radiation Safety Office was notified that a principle investigator who has recently arrived from the University of Rochester Medical Center (URMC), had vacated his previous institution without obtaining URMC Radiation Safety clearance for several pieces of equipment used in experiments that utilized radioactivity. These units are now residing safely on the CUMC campus. The Radiation Safety Office sent a technician to investigate this equipment. One tabletop centrifuge and assorted plastic containers and buckets were found. A lab member identified this equipment as the equipment that had been transported from URMC without clearance. This person was aware that the status of the equipment was an issue, because the URMC Radiation Safety Office contacted the lab directly and the technician had been expecting to hear from the CUMC Radiation Safety Office. The equipment was wipe tested and found to be clean.

Training

In accordance with regulatory requirements the Radiation Safety Office provides initial radiation safety training to all new employees of the Columbia University Medical Center and the New York State Psychiatric Institute prior to their beginning work with radiation equipment or radioactive materials. The Radiation Safety Office then provides annual refresher training.

The Radiation Safety Office also provides training in the general area of Emergency Response Preparedness. This responsibility assumed added importance as a result of the tragic events of September 11, 2001, following which the Joint Radiation Safety Committee formed a new subcommittee for the Management of Radiation Incidents. This subcommittee is chaired by David Brenner, Ph.D. and has the mission of providing the University and Hospital with professional expertise in the area of possible radiological threats and advising on the appropriate responses. As always, the Radiation Safety Office’s role in this subcommittee is to provide professional and technical personnel in order to support the Joint Radiation Safety Committee’s policies and recommendations.

In addition to providing training to outside departments and institutions, personnel within the Radiation Safety Office itself are continually undergoing training.

Significant training activities included:

1. Pursuant to Article 175 of the New York City Health Code, the following radiation safety courses and training sessions were presented from July 2003 through June 2004:
   - 12 initial training seminars for individual researchers
   - 12 annual refresher seminars for researchers
   - 12 nursing seminars for New York Presbyterian Hospital
   - Training sessions for Dental School residents
   - Training sessions for Dental Assistant students
   - Training sessions for Radiology residents
   - Training sessions for the Facilities Department.

2. For employees who could not attend the regularly scheduled classes, the Radiation Safety Office designed and implemented a self-study program, including the use of videotapes available at the Health Sciences Library. A passing grade on a quiz administered after viewing the video qualifies an employee working in Non-Human Use applications to be issued a personnel radiation dosimeter and authorizes that employee to begin work with radioactive material or radiographic equipment. If the individual's employment involves human use of radioactive material, a passing grade on the quiz results in obtaining a temporary badge until the next regularly scheduled training session is attended.

3. On February 21, 2004, the officers of the Radiation Safety Office attended a semi-annual FDNY unit drill. The drill started at 9:00 AM and lasted for about two hours. FDNY units visited radioactive storage facilities and raised questions about potential hazard of radioactive materials, especially during disasters. The drill was followed by a workshop in which Radiation Safety Office staff discussed radiation protection and emergency issues that were of concern to the FDNY units.


5. On February 26, 2004, the Officers of the Radiation Safety Office attended the 2004 Project Manager Meeting hosted by EH&S. The EH&S staff talked about the collaboration between EH&S, the Radiation Safety Office, and project managers. In this meeting, the issue of hazardous waste clearance was raised. It was emphasized that prior to a laboratory being emptied for construction, EH&S and the Radiation Safety Office (if radioactive materials involved) should be notified. The construction may not start until EH&S and the Radiation Safety Office have issued a clearance certificate.

6. On March 2, 2004, the Radiation Safety Office provided radiation safety training to PI security and housekeeping managers.

7. On April 20, 2004, the Radiation Safety Office participated in a tabletop drill conducted by the Emergency Department of New York Presbyterian Hospital. Dr. Lazar, Vice President of Medical Affairs at NYPH led this tabletop drill. Representatives of Radiation Safety, the Emergency Department, Nursing, and other departments joined in this exercise. The drill was held in the Command Center, which is in the process of being prepared for use in the event of an actual incident. The thirty participants divided into three groups, and a scenario was announced. Each group was provided with action pages, outlining the functions of individuals involved in the response. The drill scenario envisioned an accident with a chemical tanker that had exploded one mile north of the George Washington Bridge, causing multiple casualties and resulting in number victims seeking treatment at the NYPH ER. At the conclusion of the drill, the participants reviewed the response and discussed the lessons.
learned.

8. Due to recent changes in the mixed waste policy and laboratory spills, Ahmad Hatami gave refresher training for all lab managers at CUMC, NYSPI, and NYPH on April 14 and April 22, 2004. Topics discussed included Article 175, wipe tests and inventories, mixed waste, and spill procedures.

9. The Radiation Safety Office and the Emergency Room Department continue to attend meetings to discuss emergency response to a simulated terrorist attack. Since April 1, 2003, the Radiation Safety Office has been attending the Emergency Department Subcommittee meetings with regard to preparing for terrorist activities. The Radiation Safety Office ordered portable survey meters dedicated for use at the Emergency Room at New York Presbyterian Hospital. The Radiation Safety Office also has joined the ER for routine drills as well as tabletop drills.

10. On August 4, 2004 representatives of the US Department of Homeland Security, the Los Alamos National Laboratory, the Nuclear Regulatory Commission, the US Department of Energy, the New York Police Department, and the NYC Health Department visited NYPH and CUMC as a part of a voluntary cooperative demonstration project. This visit was a part of joint project called “Department of Homeland Security Radiological Threat Reduction Demonstration Project.” The purpose of this project was to undertake several actions that would demonstrate security enhancements at licensees’ sites where high-risk radioactive source material is located. A walk-through was performed by Radiation Safety Office staff and Jeannine Jennette, Director of CUMC Security, in preparation for this visit. During this walkthrough, the CUMC Radiation Safety Office was alerted to the fact that housekeeping was able to access two irradiator units that contain significant amounts of radioactive material and are capable of producing high dose rates in nearby areas. In order to ensure the safety and security of these pieces of equipment, the CUMC Radiography Safety Office and the Columbia University Security Department have required that the locks on doors to rooms in which large irradiators are housed (VC 11-223 and PH B-448) are removed from the general master key system. Access to these rooms is now restricted to the Principle Investigator, the Radiation Safety Office, and Columbia Security. The Radiation Safety Office continues to work with the US Department of Energy to accomplish secure removal and ultimate disposal of fissile and other weapons-related radioactive materials from the CUMC research inventory.


12. On August 19, 2004, Ahmad Hatami provided annual refresher training to security supervisors at the CUMC and Morningside campuses. The course included a PowerPoint presentation and was conducted to review the responsibilities of the Safety Department regarding radioactive materials and Radiation Safety Office procedures as well as the relationship between Security and the Radiation Safety Office.

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**Professional Radiation Safety and Health Physics Support**

The Radiation Safety Office provides professional radiation and health physics consultation to clinical departments, research laboratories, Authorized Users, and Responsible Investigators to ensure compliance with technical requirements in the regulations and good practice in the safe use of radioactive materials and radiation equipment.

Specific examples of professional support provided by the Radiation Safety Office in 2004 include:

In 2004, the Radiation Safety Office provided radiation safety support for 82 brachytherapy and Iodine-131 radiopharmaceutical therapy patients who received treatment from the New York Presbyterian Hospital Departments of Nuclear Medicine and Radiation Oncology. Interviews were also held with 27 outpatients scheduled for Iodine treatment. Radiation Safety Office support included: room preparation; distribution of personnel radiodiometers; performing patient and room surveys; posting instructions in patient rooms; entering instructions in patient charts; performing patient discharge surveys; room decontamination; and removing patient generated wastes for decay-in-storage and disposal.

2. Ahmad Hatami, Assistant Director of the Radiation Safety Office, participates as a member of the IACUC Animal Care Protocol Review Committee by reviewing all procedures that utilize radionuclides in animal research and reviewing other animal protocols.

3. The Radiation Safety Office participates as a member of the JRSC executive committee in reviewing all Human Use protocols using radiation.

4. The Radiation Safety Office provides training to researchers approved for human use studies to insuire compliance with all Federal, State, and City regulations and CUMC guidelines.

5. The Radiation Safety Office performs periodic audits and inspections of CUMC facilities to insuire compliance with applicable regulations and guidelines.

6. In 2004, the Radiation Safety Office performed 26 routine animal radiation surveys in the Institute of Comparative Medicine in order to minimize contamination in animal facilities and cages, protect Animal Care staff, and ensure proper disposal of animal carcasses containing radioactivity.

7. In 2004, the Radiation Safety Office provided calibration and maintenance services for 275 radiation survey instruments used throughout the Columbia University Medical Center and New York State Psychiatric Institute. The Radiation Safety Office maintains a supply of portable survey instruments available for loan to Responsible Investigators.

8. In 2004, Financial Front End, software maintained by the purchasing department, proved itself to be effective in allowing Approved Users to quickly and efficiently gain Radiation Safety Office approval when generating purchase requisitions for radioactive materials.

9. The Radiation Safety Office provides continuing radiation safety support for the Columbia University Cyclotron Facility and the Columbia University Radioligand Laboratory for the production and synthesis of PET imaging radiopharmaceuticals. This support includes: basic radiation
10. In 1997, Radiation Safety Office support for the cyclotron involved ½ FTE in personnel commitment. In 1998 and 1999 the Radiation Safety Office participated in the construction of a new PET scanning suite on the third floor of the Milstein Hospital Building. The Radiation Safety Office provided a shielding design that allows individuals to work safely in the facility throughout their pregnancy. The Radiation Safety Office obtained the licensing for this PET facility and performed the required radiation safety surveys. Similarly, in 2003 and 2004 the Radiation Safety Office reviewed the shielding design, obtained licensing, and performed the radiation safety surveys for the Kreitchman PET Center PET/CT Trailer. In addition, in 1999 and 2000 the Radiation Safety Office, as required by the New York State Department of Environmental Conservation, prepared a complete revision of the Columbia University Medical Center Radiation Control Permit and at NYSDEC direction implemented a program to monitor, quantify, and report on atmospheric discharges of radioactive materials from the Cyclotron, Radioligand Laboratory, and PET Suites. By 2004, the production of radioisotopes by the cyclotron facility has increased four-fold (4X) over 1999/2000 levels, and Radiation Safety Office support for cyclotron, radioligand, and PET as required by regulatory agencies including NYSDEC and NYC DOHMH has grown to more than 2 FTE in personnel coverage.

The Radiation Safety Office is participating in the planning of and provides support for the construction and operation of a new NYSTAR funded PET research center. This center will be operated by Columbia University Medical Center rather than an outside vendor and will include two cyclotrons, a radiopharmacy, a radioligand synthesis laboratory, PET, PET/CT and PET/MRI scanning suites, and an extensive micro-PET and mini-PET animal facility. Support for this project will require a significant expansion of the Radiation Safety Office. Radiation Safety office support for this project will include, but not be limited to, the following projects in addition to current levels of support:

- Obtaining permits to allow Columbia University Medical Center to decommission the existing RDS-112 cyclotron facility in the Milstein Hospital Basement and transport the neutron activated cyclotron components and the existing pneumatic transport system to the School of Public Health. Radiation Safety Office staff will be required to monitor the decommissioning and the transport of the neutron activated cyclotron components.
- Professional radiation safety review of plans, shielding design, air exhaust systems, safety systems, pneumatic transport systems, and proposed radiation safety policies and procedures for the entire NYSTAR facility and the formulation of reports to the Joint Radiation Safety Committee.
- Obtaining for Columbia University Medical Center the necessary New York City Department of Health Radioactive Materials Licenses and amendments to the New York State DEC Radiation Control Permit for the construction and operation of the NYSTAR facility. This involves preparing and submitting the calculation of expected public and personnel radiation doses resulting from their operation, including those resulting from radioactive discharges to the atmosphere.
- Performing extensive radiation safety surveys of the newly constructed facilities prior to their being placed into operation, including: the two cyclotrons, the radiopharmacy and radioligand laboratories, and the human-use and animal scanning suites. In addition, the Radiation Safety Office will need to implement an ongoing program for the continuous monitoring and quantification of discharges of radionuclides to the atmosphere.
- Continuing periodic audits and inspections of all the NYSTAR research facilities placed into service to ensure compliance with regulatory requirements, personnel safety, and Columbia University Medical Center policies and procedures.

11. The Radiation Safety Office continues the process of scanning paper documents into the PaperPort software application. This process is a measure to back-up our paper files with digital copies that may be transferred to CD-ROM or DVD formats. This electronic document system allows for quicker search and retrieval of information and reduces the quantity of paper documents maintained in the RSO files. During the move to our new location, we have reviewed files to determine which are to be retained, archived, or disposed of after they are converted into digital files.

12. The Radiation Safety Office provided consultation in the preparation of a grant application for the development of a “Center for Medical Countermeasures against Radiation” (CMCR). The National Institute of Allergy and Infectious Disease is providing $25 million in research grants to establish 5 CMCRs throughout the nation. The goal of these centers is the development of new medical products that will protect against, mitigate, and treat the consequences of radiation exposure from terrorist attacks.

**Professional Radiation Safety and Medical Physics Support for Non-Radiology X-ray Activities**

The Joint Radiation Safety Committee in agreement with New York Presbyterian Hospital has assigned the Radiation Safety Office responsibility for Radiation Safety and Medical Physics support for those clinical facilities outside the Department of Radiology that use x-ray equipment. The two major components of this program are the quality assurance program for dental radiography and the audit program for facilities outside Radiology and Dentistry that utilize x-ray equipment in Human Use applications.

The dental quality assurance program is designed to optimize the radiological safety and clinical quality of dental radiography. This program is based on recommendations for quality assurance that have been promulgated by a number of professional organizations, including the National Council on Radiation Protection and Measurements (NCRP), the Bureau of Radiological Health of the Food and Drug Administration, the American College of Radiology (ACR), and the American Academy of Dental Radiology Quality Assurance Committee. In this program, the Radiation Safety Office...
Office has primary responsibility for preliminary radiation safety shielding evaluation, acceptance testing, diagnostic quality assurance, and radiation safety surveys on all dental x-ray units installed at the following locations:

- Morningside Dental Associates: 9 intraoral units, and 1 panoramic - cephalographic unit at two locations
- Ambulatory Care Networked Corporation (ACNC): 2 intraoral units and 1 panoramic–cephalographic unit
- Babies Hospital OR: 1 portable intraoral unit
- Vanderbilt Clinic Teaching & Research Areas: 1 panoramic unit, 1 panoramic–cephalographic unit, 23 intraoral units, and 1 intraoral–cephalographic unit
- Dentcare Clinic (Intermediate School 183): 1 intraoral unit
- New York State Psychiatric Institute: 1 intraoral unit and 1 panoramic unit
- Columbia Eastside: 6 intraoral units and 1 panoramic – cephalographic unit
- Columbia North: 5 intraoral units and 1 panoramic unit
- Mobile Dental Facility: 2 intraoral units
- Mannie L. Wilson Health Care Center: 5 intraoral units and 1 Panoramic Unit.

The Radiation Safety Office and Radiology Medical Physics jointly run the audit program for x-ray facilities that operate outside of Radiology and Dentistry. This program is conducted in accordance with the conditions of New York City X-ray Permit No. H96007635386, as specified in Article 175 of the New York City Health Code. The Radiation Safety Office maintains this license so that x-ray equipment may be utilized in human use applications throughout CUMC. In this audit program, the Radiation Safety Office is primarily responsible for ensuring that each site follows the proper safety practices and keeps the proper records, while Radiology Medical Physics is responsible for performing all technical tests. The following locations are audited under this program:

- Urology Department, Atchley 11th Floor: 1 Fluoroscopy unit
- Endoscopy Department, Atchley 13th Floor: 3 C-Arm Fluoroscopy units
- The Spine Center, Neurological Institute, 5th Floor: 1 C-Arm unit
- Sports Medicine, Dodge Fitness Center/Bakers Field: 1 Mini C-Arm unit
- Cystoscopy Suite, Milstein 4th Floor: 3 Radiographic/Fluoroscopic units
- Cardiac Care, Milstein 5th Floor: 1 C-Arm unit
- Presbyterian Hospital 5th Floor: 1 C-Arm unit
- Harkness Pavilion 9th Floor: 1 Bone Densitometer

In 2004, the Radiation Safety Office provided the following support of the above programs:

1. The Radiation Safety Office upgraded its Dental QA equipment, the RTI Barracuda system, in order to allow for more accurate measurement of the absorbed dose received from panoramic dental x-ray machines.
2. Bruce Emmer gave the annual Fellows Lecture to the radiology residents.
3. James Dolan was trained to perform audits and completed audits of several of the Non-Radiology facilities.

### Radiation Safety Office Personnel and Facilities

Significant personnel and facilities activities included:

1. On February 1, 2004, Olga Loukhton resigned from her post as Chief Technician.
2. On July 1, 2004, Shuntong Guo resigned from his post as Junior Physicist.
3. In January 2005, Mutian Zhang announced his intention to resign from his post as Junior Physicist, effective February 17, 2005.
4. In 2004, the Radiation Safety Office reevaluated the cost sharing arrangement between CUMC, NYPH and NYSPI for the RSO Main Account (2-50126).
5. In the calendar year 2004, Gerald Fischbach, M.D., Dean of Columbia University Medical Center, directed the JRSC to create a position for a Radiological Quality Assurance Officer reporting to the Radiation Safety Officer. The incumbent in this new position will be responsible for assuring that investigations involving human subjects, approved by the IRB, RDRC and/or the JRSC for the use of radioactive materials and/or x-rays, comply with all regulatory requirements, regulatory and professional guidelines, and institutional policies and procedures. The incumbent will provide oversight to the production and quality control of isotopes and radio-labeled compounds used for clinical studies and for human subject protocols approved by either the Joint Radiation Safety Committee or the Radioactive Drug Research Committee. This is a professional position requiring an advanced degree and appropriate certification and licensure. This position is in support of human subject research that brings to the institution several million dollars in grants and contracts each year. Most importantly, filling this position expeditiously will serve as a part of the corrective actions taken by CUMC in response to the ongoing FDA audit/investigation.
6. Due to the resignations discussed above, the Radiation Safety Office currently has several vacant positions. On October 29, 2004, the Radiation Safety Office received a letter from Markus Spivak, the Environmental Radiation Specialist at the New York Department of Energy Conservation. This letter expressed concern regarding the current staffing level of the Radiation Safety Office and noted that it is “extremely important to have adequate radiation safety staffing.” The letter also strongly recommended that these positions be filled “with all due haste.”
7. James Dolan was promoted from Technician B to Junior Physicist on December 1, 2004.
8. Dae In Kim was promoted from Junior Physicist to Health Physicist in January of 2005.
9. In response to the concerns of the NYSDEC over our staffing levels, with the assistance of Dr. Lewy the Radiation Safety Office filled the following vacant positions:
   - Radiation Protection Supervisor: The Radiation Protection Supervisor position was posted, and we interviewed suitable candidates. We were able to finalize the selection of Shinkyu Park. Mr. Park began work for the Radiation Safety Office on March 21, 2005.
   - Chief Technician: The Chief Technician position was posted, and candidates were interviewed. We were able to finalize the selection of Dong Michelle Kang.
Members of the Radiation Safety Office staff in the above scenes are participating in a dirty bomb preparedness drill.

Salmen Loksen on the Milstein Hospital building rooftop inspecting the emission stack for the Radioligand Laboratory.

The new Columbia/Kreitchman PET/CT trailer located in front of 722 W. 168th Street. The Radiation Safety Office assisted with shielding design and regulatory issues.
AMUNDSON, SALLY A., Sc.D.
Adjunct Faculty
  NCI Radiation Epidemiology Branch, National Institutes of Health, Adjunct Investigator
Member
  Radiation Research Society, Program Committee
  National Council on Radiation Protection and Measurements (NCRP)
Reviewer
  Cancer Research
  Cancer Research
  Carcinogenesis
  Genomics
  Molecular Biology of the Cell
  Molecular Cancer Research
  Molecular Cancer Therapeutics
  Mutation Research
  Radiation Research
Honors
  Michael Fry Research Award of the Radiation Research Society

BALAJEE, ADAYABALAM S., Ph.D.
Member
  American Association for Advancement of Science
  Radiation Research Society
  Indian Association of Radiation Biology
Reviewer
  Nucleic Acids Research
  Advances in Space Research
  Medical Science Monitor
Honors
  Edited a book on “DNA repair and human diseases” for Landes Biosciences, Texas, USA

BIGELOW, ALAN, Ph.D.
Member
  American Physical Society
  Radiation Research Society
Reviewer
  Nuclear Instruments and Methods in Physics Research B
Student Mentoring
  New York City Stuyvesant High School summer student apprenticeship and project for the Intel Science Competition

BRENNER, DAVID J., Ph.D., D.Sc.
Member
  Columbia University Radiation Safety Committee, Chairperson
  National Council on Radiation Protection and Measurements (NCRP)
  Radiation Research Society, Program Committee
  International Congress on Radiation Research, Program Committee
  TV and radio appearances on the subject of full body CT examinations
Editorial Work
  Radiation and Environmental Biophysics, Assoc. Editor

CALAF, GLORIA M., Ph.D.
Adjunct Faculty
  University of Tarapaca; Faculty of Sciences; Dept. of Biology and Health, Arica, Chile, Full Professor
Member
  Biology Society of Chile
  Mastology Society of Chile
  Chilean Society of Citology
  Chilean Society of Cancer
  New York Academy of Sciences
  Tissue Culture Association
  International Association of Breast Cancer Research
  American Association of Cancer Research
  Society of Experimental Biology and Medicine
  Radiation Research Society
Student Mentoring
  Autonomous University of Madrid, Spain, Ph.D. Advisor
Teaching
  University of Tarapaca, Coordinator: “Individual predisposition to Cancer and treatment”, January 5th to January 12th, 2004
Reviewer
  Grant:
  Fondo de Desarrollo Científico y Tecnológico (Fondicyt)
  Manuscript:
  British Journal of Cancer
  Mutation Research
  International Journal of Radiation Oncology Biology Physics
  International Journal of Radiation Biology
Grants
  Fondecyt 1040300, scholar grant
  UTA 4714-04, scholar grant

GEARD, CHARLES R., Ph.D.
Member
  American Society of Therapeutic Radiology and Oncology (ASTRO)
  Environmental Mutagen Society
  Advisory Committee on Radiobiology, Brookhaven National Laboratory
  Scientific Review Panels, Research Council, Ontario, Canada
Editorial Work
  International Journal of Radiation Biology, Editorial Board
Reviewer
  British Journal of Cancer
  Mutation Research
  Radiation Research
  Mutagenesis
Student Mentoring
Columbia University, resident in Radiation Oncology

HALL, ERIC J., D.Phil., D.Sc., FACR, FCR
Member
Royal College of Radiology
British Institute of Radiology
American Board of Radiology, Radiotherapeutic Written-Test Committee
American Society of Therapeutic Radiology and Oncology (ASTRO)
Radiation Research Society
American Radium Society
International Association of Radiation Research, Past President
Columbia University, Herbert Irving Comprehensive Cancer Center, Director, Radiation Physics and Biology Program
Columbia-Presbyterian Medical Center, Joint Radiation Safety Committee, Chairman; Radioactive Drug Research Committee, Chairman
National Council on Radiation Protection and Measurements, Committee 1, Emeritus Member

Editorial Work
Intl Journal of Radiation Oncology Biology Physics, Editorial Board
International Journal of Brachytherapy

HEI, TOM K., Ph.D.
Adjunct Faculty
Department of Radiological and Environmental Health Science, Colorado State University, Fort Collins, Co., Adjunct Professor
Department of Ion Beam Bioengineering, Chinese Academy of Sciences, Hefei, China, Adjunct Professor and Doctorate Student Mentor
University of Hong Kong, External Examiner
Member
NIH Pathology C Study Section, Chairman, Ad Hoc Review Panel
NCI Cancer Etiology Study Section, Chairman, Ad Hoc Review Panel
NCI Division of Cancer Biology, Panel Member
EPA Expert Panel on Asbestos Toxicology
American Association for Cancer Research, Annual Meeting Program Committee
Department of Med. Faculty Recruitment Committee
Radiation Research Society
Environmental Mutagen Society
Oxygen Society

Students Mentoring
Doctoral Students of Environmental Health Sciences, Columbia University, School of Public Health
New York City High School Science Students for Intel Science Project
Faculty Advisor for Exchange 4th year Chinese Medical Students from Fudan University

Reviewer
Proceedings of the National Academy of Sciences

Cancer Research
Carcinogenesis
Radiation Research
Environmental Health Perspective
International Journal of Radiation Biology
Cancer Letters
Book proposal for CRC
Book proposal for Kluwer Academic Publishers

Editorial Work
Advances in Space Sciences, Section Editor

LIEBERMAN, HOWARD B., Ph.D.
Member
Summer Research Program for NYC Secondary School Science Teachers, Columbia University, Advisory Board
Israel Cancer Research Foundation, Scientific Advisory Board
Columbia University College of Physicians and Surgeons, Faculty Council
American Association for the Advancement of Science
American Society for Microbiology
Environmental Mutagen Society
Genetics Society of America
Sigma Xi
Theobald Smith Society

Reviewer

Grants:
Basic and Preclinical Subcommittee C of the NCI Initial Review Group, Member
NIH Special Emphasis Panel, Ad Hoc Review Panel
Joint Center for Radiation Therapy Foundation, Harvard Medical School, Ad Hoc Review Panel
Postdoctoral Fellowship panel, Israel Cancer Research Foundation.
Pennsylvania Department of Health

Manuscripts:
Cancer Research
Intl Journal of Radiation Oncology, Biology and Physics
Mutation Research
Radiation Research

MARINO, STEPHEN A., M.S.
Member
Columbia University Radiation Safety Committee
Radiation Research Society

MITCHELL, CATHERINE, Ph.D.
Member
Radiation Research Society

Reviewer

Radiation Research
Advances in Space Research

Awards
The Gilbert Beebe Award for outstanding scholar-in-training in the field of radiation epidemiology, Radiation Research meeting, St. Louis, MO, 2004
YIN, YUXIN, M.D., Ph.D.  
Member  
Radiation Research Society  
Radiation Research Society Scholars-In-Training Committee  
Reviewer  
Radiation Research  
Advances in Space Research

PONNAIYA, BRIAN, Ph.D.  
Member  
Radiation Research Society  
Reviewer  
International Journal of Radiation Biology  
Radiation Research  
Oncogene

YIN, YUXIN, M.D., Ph.D.  
Adjunct Faculty  
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Member  
American Association for Cancer Research  
Students Mentoring  
Advisor for a Ph.D. student in the Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University  
Reviewer  
Cancer Research

ZHAO, YONGLIANG, Ph.D.  
Member  
Radiation Research Society  
American Association for Cancer Research  
Grants  
RSNA Research Seed Grant  
NIEHS Center Pilot Grant  
NASA Project Grant

### Publications

19. Hall EJ. Henry S. Kaplan Distinguished Scientist Award 2003. The crooked shall be made straight; dose-response relationships for carcinogenesis. Int J Radiat ...


