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LAURISTON S. TAYLOR LECTURE ON RADIATION PROTECTION AND MEASUREMENTS: WHAT MAKES PARTICLE RADIATION SO EFFECTIVE?

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Abstract

The scientific basis for the physical and biological effectiveness of particle radiations has emerged from many decades of meticulous basic research. A diverse array of biologically relevant consequences at the molecular, cellular, tissue, and organism level have been reported, but what are the key processes and mechanisms that make particle radiation so effective, and what competing processes define dose dependences? Recent studies have shown that individual genotypes control radiation-regulated genes and pathways in response to radiations of varying ionization density. The fact that densely ionizing radiations can affect different gene families than sparsely ionizing radiations, and that the effects are dose- and time-dependent has opened up new areas of future research. The complex microenvironment of the stroma, and the significant contributions of the immune response have added to our understanding of tissue-specific differences across the linear energy transfer (LET) spectrum. The importance of targeted vs. nontargeted effects remain a thorny, but elusive and important contributor to chronic low dose radiation effects of variable LET that still needs further research. The induction of cancer is also LET-dependent, suggesting different mechanisms of action across the gradient of ionization density. The focus of this 35th Lauriston S. Taylor Lecture is to chronicle the step-by-step acquisition of experimental clues that have refined our understanding of what makes particle radiation so effective, with emphasis on the example of radiation effects on the crystalline lens of the human eye.

Keywords

National Council on Radiation Protection and Measurements (NCRP); particle physics; relative biological effectiveness (RBE); health effects; award presentation

INTRODUCTION

It is extremely humbling to have been named the 35th Lauriston S. Taylor Lecturer in 2011, and I am very grateful to the National Council on Radiation Protection and Measurements (NCRP) for this huge honor. Although I never had the privilege to meet Dr. Taylor, I had long been aware of his major contributions to the field of radiation sciences. I knew he was

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Dedication—This paper is dedicated to my husband George Zizka and our daughter Maria Zizka.

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called “Mr. Radiation Protection” for his brilliant, groundbreaking leadership in standardizing radiation measurements and safety at the national and international level. He served on both the International Commission on Radiological Protection (ICRP) and the International Commission on Radiation Units and Measurements, and was the founder and the first president of NCRP in 1929. In a manuscript copyrighted in 1990 entitled “What you need to know about radiation,” Dr. Taylor captured my interest when he discussed issues related to radiation protection at the personal and family levels, as well as issues related to the importance of making reasonable social and political choices (ISU 2012).

In this lecture, I would like to extend Dr. Taylor’s 1990 treatise to address “What you need to know about *particle* radiation”. I will review the underlying mechanisms of action of particle radiation that make them so effective based on the perspective of 36 y of experience in the laboratory and working with physicians in the clinic, with emphasis on the example of particle radiation effects on the crystalline lens of the human eye. I will describe how our understanding has grown through the years, and what research in my opinion is still needed to further our understanding of charged particle radiobiology.

Radiation is energy in the form of waves or particles. A photon is a particle of electromagnetic (EM) radiation that is both a particle and a wave, but a “charged particle” is a particle with an electric charge that may either be a subatomic particle or an ion. Particle radiation is referred to as a “particle (or ion) beam,” if the particles are all moving in the same direction, similar to a light beam. In today’s world, cancer patients are being treated with charged particle beams of protons or carbon (Blakely and Chang 2012), and astronauts traveling in space are exposed to galactic cosmic rays and solar particle events that include many different particle radiations (Cucinotta and Chappell 2010).

A diverse array of biophysical processes exists across the EM spectrum that underlies differences in energy absorption and biological effects depending on radiation wavelength and frequency. On Earth, particle accelerators use EM fields to propel well-defined charged particle beams to high velocities in a spiral trajectory, while the sun and cosmos provide unpredictable complex fields of particle radiations in outer space. Charged particles therefore represent the most energetic extreme of the EM spectrum, whether encountered in the clinic or in space travel. The stopping ion beam depth-dose energy deposition Bragg peak profile demonstrates significant differences in energy absorption compared to conventional or particle ionizing radiations that are lower on the EM scale, depending on the particle atomic number, velocity, dose, and dose rate of the exposure.

PARTICLE PHYSICS

The passage of a stopping charged particle across absorbing material such as tissues of the body leaves behind a trail of molecular changes. “Track structure” is the spatial and temporal organization of atomic and molecular events that result from the interaction of charged particles with matter. In an accelerator, individual charged particles stripped of their electrons can be produced as ion beams at energies of several hundred MeV amu⁻¹ having a range of absorption sufficient to penetrate a human body. At high energy, the tracks created by the ions in film emulsion reveal a dense, tight cross-sectional “core” caused largely by glancing collisions, and a “penumbra” which is due to energetic knock-on collisions. In contrast, at the stopping low energy range of the track, the cross-sectional track structure is limited to a tight core of ionization (Tobias 1979). Chatterjee et al. (1973) have calculated the yield of different chemical species for aqueous systems in the core and penumbra, and the subsequent diffusion of free-radical density distributions modifying the track structure with time. Different chemical species produced by the physical absorption of energy in

aqueous materials results in biochemical changes in the absorbing material, such as DNA (Chatterjee and Holley 1993).

Track structure models describe the relationship between the spatial distribution of energy deposition in the form of ionizations and excitations in the geometric structure of target molecules. Some of these theoretical models such as the MOCA14 (Wilson et al. 1984; Charlton et al. 1985) and PITS (Stewart et al. 2002; Mainardi et al. 2004) codes, consider the stochastics of ion tracks in evaluating frequency distributions, but have been limited to dealing with maximum energies of 5 MeV amu^{-1} and have not been extended to high atomic number and energy ions. A deterministic model of frequency distributions for total energy deposited in small volumes similar to DNA molecules from high-energy ions of interest for cancer therapy and other applications has been developed (Cucinotta and Dicello 2000). This model predicts that at high energies, the lateral extension of an ion's track, denoted as the track width, will extend to several hundred micrometers, or even a few millimeters due to delta-ray transport. Several new approaches to modeling track structure effects have been published for evaluation of particle effects in cancer therapy and in space travel to tackle remaining challenges to accurately and completely characterize particle interaction physics (Sato et al. 2009, 2012; Plante and Cucinotta 2011; Russo et al. 2011a, 2011b; McNamara et al. 2012; Toburen 2012).

Linear energy transfer (LET) is the linear rate of loss of energy by an ionizing photon or charged particle traversing a medium, usually reported in units of $\text{keV } \mu\text{m}^{-1}$. Below $\sim 10 \text{ keV } \mu\text{m}^{-1}$, EM radiation results in what is referred to as "low-LET" radiation, whereas above that threshold is considered "high-LET" radiation. The LET of an individual particle track changes as the particle stops in matter. The LET can be calculated in several ways, for example by track averaging, or by dose-averaging, or by calculating the unrestricted linear collision stopping power in water, the LET_∞ . Since primary particle beams can interact with absorbing material in several types of collisions, secondary fragments are produced which complicate the calculation of the overall effective LET. Tertiary events can also be considered in more precise estimations of ionization and excitation events. One major issue that is often not appreciated is that two particle beams of different atomic number at the same LET value can have very different track structure and fragmentation contributions. This fact has hampered the full usefulness of the LET concept in describing a biological effect since it does not adequately, nor uniquely describe in a single term all of the features of radiation quality. The LET of a particle is dependent on particle Z and E, and the fragmentation components from secondary collisions.

BIOLOGICAL EFFECTIVENESS OF CHARGED PARTICLE DOSE

A hierarchy of ion beam inactivation cross sectional effects has been reported in a variety of organisms covering over eight logs from 10^{-14} cm^2 to 10^{-6} cm^2 as a function of LET in units of $\text{MeV cm}^2 \text{ g}^{-1} \text{ tissue}$ (Blakely 1992) (Fig. 1). The data appear to depend on the physical dimensions of the radiation-sensitive biological targets. Results of high-LET effects using a variety of endpoints in in vitro systems, including single- and double-strand DNA breaks, cell-cycle delays, chromosome aberrations, mutation induction, and cellular and DNA repair have been reported (Blakely et al. 1984). However the LET-dependence of many diverse cell lines demonstrates a maximum RBE for cell killing near $150 \text{ keV } \mu\text{m}^{-1}$ for cell studies in different positions of pristine Bragg peaks or in different positions of beams with "extended" or spread-out Bragg peaks (Fig. 2). This diversity in cellular radiosensitivity in vitro can of course lead to variable biological effectiveness for a fixed physical dose profile across a spread-out Bragg curve. It has been noted that cells of lymphoid origin that are susceptible to apoptotic death appear to have a maximum RBE at a

lower LET value (at $\sim 60 \text{ keV}\mu\text{m}^{-1}$), than other types of cells that die a necrotic death from radiation (Blakely et al. 1984).

Radiation is a supreme oxidative damaging agent, but if oxygen is removed from the system, the biological systems sustain less oxidative damage and are thus more radioresistant. The “oxygen effect,” for example the difference between cell killing under hypoxic conditions vs. under aerobic conditions, can be of the order of threefold for low-LET radiations such as x rays or gamma rays. Damage from the high ionization density of high-LET radiation sources is less susceptible to the dependence on the presence of oxygen. Thus the oxygen effect is reduced to a very low level near one at high-LET values greater than $150 \text{ keV}\mu\text{m}^{-1}$. This feature of high-LET radiations has been considered very important to the eradication of large tumors that frequently outgrow their oxygen supply and become hypoxic. More recent research has revealed that the hypoxic status of a cell is associated with a unique signal transduction pathway that involves proteins that confer radioprotection (Ji et al. 2010). It is possible to titrate out the low-LET dose component that is dependent on oxygen in a mixed-LET beam using radical scavengers such as DMSO (Chapman et al. 1979), or hypoxic cell radiosensitizers (Chapman et al. 1978).

The growth status of a cell culture is another significant variable to radiation responsiveness. Dividing cells are in general more sensitive to radiation than cells in stationary growth (Bergonie and Tribondeau 1906). Each phase of the cell cycle is known to have variable radiosensitivity to low-LET radiations. In general there are two “peaks” of low-LET resistance, one in G1-phase, and the other in late S-phase (Sinclair and Morton 1965). High-LET radiation quantitatively reduces the amplitude of the wave of cell-cycle-dependent radioresistance, and qualitatively alters the mitotic/early G1-phase response (Blakely et al. 1985), and can lead to drastic G2 arrest (Lucke-Huhle et al. 1979).

Particle-radiation effects are characterized by enhanced DNA damage and reduced repair efficiency (Roots et al. 1979), reduced capacity for molecular scavenging of reactive oxygen species (Roots et al. 1982), and production of short DNA fragments (Rydberg et al. 1998a; Rydberg 2001). Particle damage is notably “clustered” (Goodwin et al. 1994; Rydberg et al. 1998b) and more susceptible to misrepair (Goodwin and Blakely 1991, 1992). The lack of checkpoint control (Lucke-Huhle et al. 1979) leads to a shift in chromosomal aberrations (Iliakis et al. 2004; Nasonova et al. 2004). Recent studies in radiation-induced foci with heavy-ion microbeams have provided intriguing new information as to the sequence of DNA damage and repair proteins following the passage of a particle track (Tobias et al. 2010; Jakob et al. 2011). Repair of DNA damage induced by accelerated heavy ions has recently been reviewed (Okayasu 2012).

WHY INTEREST IN PARTICLE THERAPY FOR CANCER?

There are many reasons why charged particles were considered by Robert Wilson in 1946 (Wilson 1946) to be ideal for use in cancer radiotherapy, and expanded on by later experiments of many others. Precise physical depth-dose profiles allow treatment plans that conform the dose to the tumor and minimize the dose to surrounding normal tissues. Subsequent studies of particle radiobiology revealed increased DNA damage to tumors, especially hypoxic tumors relative to conventional tumors (Barendsen et al. 1966; Todd 1967), less repair of sublethal and potentially lethal damage throughout all phases of the cell cycle (Elkind and Whitmore 1967), and most recently the concept that the overall treatment time can be reduced with charged particles has many advantages to the patient and the clinical throughput of patients (Tsuji et al. 2007; Okada et al. 2010).

The clinical accomplishments of particle therapy with protons, helium, and heavier ion beams continue to be reported (Linstadt et al. 1990; Debus et al. 2000; Slater 2007; Combs

et al. 2010a, 2010b; Okada et al. 2010; Suit et al. 2010). Early tumor sites successfully treated were limited to the head and neck, sarcomas of bone and soft tissues, and prostate, but recent progress has been made with a much larger number of tumor locations including lung, liver, uterus, rectum, esophagus and pancreas.

PARTICLE TREATMENT OF EYE TUMORS

Uveal melanoma tumors of the eye involve the iris, ciliary body, or choroid (collectively referred to as the uvea). Tumors arise from the pigment cells (melanocytes) that reside within the uvea giving color to the eye. These melanocytes are distinct from the retinal pigment epithelium cells underlying the retina that do not form melanomas. Uveal melanoma was one of the first successfully particle-treated tumor sites, initially with protons in Boston (Gragoudas 1980, 2006), and then with helium ions in Berkeley (Saunders et al. 1985; Char 1989). Precise helium high-dose radiotherapy of uveal melanoma eradicated 97% of the tumors with the patients retaining their eyes with vision (Linstadt et al. 1988), and few surrounding tissue toxicities (e.g., neovascular glaucoma) (Linstadt et al. 1990). Only patients with large anterior lesions developed late-appearing metastases, primarily to the liver (Nowakowski et al. 1991). The brain behind the eye received virtually no radiation dose, something difficult to accomplish with conventional radiotherapy, but most of the patients developed a radiation-induced cataract since the dose to the retinal tumor was delivered through the crystalline lens (Meecham et al. 1994). The dose to the lens was subsequently split in half and delivered in two treatment ports through the tough white sclera (Daftari et al. 1997), with a concomitant decrease in the incidence of cataract. Currently University of California, San Francisco radiation oncologists are using 56 GyE (gray-equivalent or the dose weighted for RBE) of protons in four fractions (in 4 d total) at the University of California, Davis accelerator to treat uveal melanoma[†] (Desjardins et al. 2012).

TYPES OF CATARACTS

Radiation cataract is a late-appearing effect of radiation (Worgul et al. 1996), with many parallels to radiation-induced cancer. In the past, carcinogenic effects of radiation were assumed for purposes of radiation protection to have no threshold, and to behave as stochastic phenomena; whereas radiation cataract was thought to require the killing of cells and thus was considered to be a deterministic effect dependent in severity with the extent of cell loss, and with a threshold of detectability which depended on the sensitivity with which the consequences of cell loss could be measured (Upton 1987). Currently the understanding of radiation-induced cataracts has changed and protection guidelines are being reconsidered (ICRP 2007, 2011). The human crystalline lens is known to be a radiosensitive tissue that responds with opacification in a delayed time course depending on the radiation type and exposure level (Merriam and Focht 1957; Merriam et al. 1972). Opacification can be due to misfolding of the crystalline proteins (Benedek 1997) or due to misregulation of lens cell morphology. Cataracts are degenerative lesions that progressively change and increase in size, and can be defined in different ways, such as minor lesions not affecting sight, or as major lesions affecting vision. It is interesting to note that cancer of the lens of the eye has never been reported (Kleinstein and Lehman 1977). In contrast, the lens appears to respond to cancer-producing agents by becoming opaque.

Cataracts appear in some idiopathic or congenital conditions in young people (Trumler 2011), and in most of us in the sixth or seventh decade of life related to aging (Michael and Bron 2011). In fact, there are four main types of age-related cataracts:

[†]Mishra K. Private communication

- “nuclear cataract” affect the center of the lens and have been related to smoking;
- “cortical cataract” affect the edges of the lens and have been linked to diabetes and excess nonionizing ultraviolet B light exposure;
- “posterior subcapsular cataract (PSC)” affect the back part of the lens and have been linked to steroids, diabetes, and exposure to ionizing radiation (Robman and Taylor 2005); and
- the most recently identified cataract type: “supranuclear cataract” affect the area just above the center of the lens and have been linked to Alzheimer’s disease (Goldstein et al. 2003).

Alzheimer’s disease lenticular opacification was found to be related to the accumulation of beta amyloid protein, and was linked to Down’s syndrome (Moncaster et al. 2010). The Down’s syndrome cataract has been shown to be associated with trisomy of chromosome 21; the human beta amyloid gene is located on chromosome 21 and its triplication accelerates the accumulation of the beta amyloid protein.

The lens has several anatomical regions. The “capsule” isolates the lens from vascularity and from other organs in the body (Fig. 3). The anterior single cell layer of “lens epithelial cells” differentiates and the cells migrate into a region of “elongating fiber cells” at the bow on each side of the body of the lens. These fiber cells continue to compact toward the interior to become mature denucleated “cortical fiber cells” surrounding the internal “nucleus” of the lens that continues to develop from embryogenesis into adulthood (Taylor et al. 1996).

Lawrence Berkeley National Laboratory (LBNL) spearheaded an effort to understand more about the particle dose distribution to the lens that was associated with the delayed appearance of cataract (Meecham et al. 1985) in uveal melanoma patients. By deconvoluting the isodose treatment profiles, and analyzing the dose distribution to the body of the lens, LBNL learned that the proportion of patients with cataract correlated with the percentage of lens anterior segment in the radiation field (Fig. 4). The percentage of cataracts varied depending on the anatomical distance of the lens from the retinal lesion. For patients whose tumors were more posterior in the eye and farther away from the lens, the doses were smaller and associated with less risk of cataract than the case for patients with anterior tumors close to the lens.

LBNL was intrigued with the question, “why do opacifications form in different anatomical locations in the lens”? The literature indicated that antioxidants are unevenly distributed in the lens (Sweeney and Truscott 1998; Lim et al. 2007; Xing and Lou 2010), that the water diffusion system of the lens constantly redistributes small molecules (Moffat et al. 1999; Moffat and Pope 2002; Schachar et al. 2008), and that regions of the lens have diverse gap junction coupling channels that facilitate fluid circulating through the lens (Gao et al. 2011). There is also evidence for regional distribution of glutathione in different forms of human cataract (Pau et al. 1990). The content of glutathione is high in the anterior lens cortex and epithelium and in the posterior lens cortex and does not decrease with age. Glutathione content is substantially lower in the lens nucleus and in supranuclear cataract. The subcapsular cataract shows a rapid and pronounced progressive decrease in glutathione content. The molecular mechanisms underlying changes in radiation sensitivity of each of these lenticular compartments remains to be clarified.

RADIATION CATARACT: MORPHOLOGICAL OBSERVATIONS

Radiation-induced precataractous cellular changes had been reported in human and animal lens studies in a time course after radiation exposure (Cogan and Donaldson 1951; Cogan et al. 1952; Upton et al. 1953; Christenberry et al. 1956; Fujinaga 1973; Kobayashi and Suzuki 1975; Hayes and Fisher 1979; Lett et al. 1980, 1991; Rini et al. 1983, 1986; Yang and Ainsworth 1987; Riley et al. 1991; Worgul et al. 2007). Early histology revealed mitotic arrest of the germinative epithelial cells, followed by nuclear fragmentation and extrusion, and broadening of the nuclear bow with the appearance of abnormal mitoses. Anterior cortical clefts appeared and granular “dots” followed the line of fiber cells. Abnormal fiber cells migrated toward the posterior pole of the lens. Fiber cell swelling and interfibrillar clefts appeared. Multiple posterior subcapsular opacities appeared due to the posterior displacement of abnormal epithelial cells. The cataractous lesions progressed in terms of total area and volume as a granular white opacity, and depending on the location relative to the visual axis, finally led to blindness unless treated with cataract surgery and replacement of the opaque natural lens with a plastic lens.

Cataract appearance after radiation exposure is dependent on many key physical, chemical and biological variables, including the radiation type, dose level, and dose rate (Christenberry et al. 1956), dose fractionation regime (Worgul et al. 1989; Brenner et al. 1996), the species (Christenberry et al. 1956; Lett et al. 1991; Cox et al. 1992; Niemer-Tucker et al. 1999); and genetic background (Worgul et al. 2002, 2005; Hall et al. 2005; Kleiman et al. 2007) of the exposed organism, its age at irradiation (Hanna and O’Brien 1963; Michel et al. 2011) and gender and hormonal status (Bigsby et al. 2009; Henderson et al. 2009) at exposure, its life-span (Lett et al. 1991), and the organism’s diet (Delcourt 2007; Davis et al. 2010) and exposure to certain drugs (Vogel et al. 2006; Wang et al. 2009a; 2009b).

Animal studies of radiation cataract are numerous, but frequently difficult to compare for a number of reasons. The cataract-scoring systems cannot be easily normalized due to the diverse numerical scoring systems with different full ranges, the somewhat subjective use of various cataract morphological descriptors, and the difficulty in extrapolating the time-course of radiation-induced human cataract from animal models with diverse life spans and genetic backgrounds (Lett et al. 1989).

RADIATION CATARACT: HUMAN EXPOSURES AND DOSE LIMITS

Radiation-induced cataract has been investigated in a number of human radiation exposure situations related to occupational or accidental exposures (Worgul et al. 2007; Wakeford 2009a; 2009b; 2009c) clinical treatments with radiation for disease or medical conditions (Deeg et al. 1984; Ozsahin et al. 1994; Belkacemi et al. 1996, 1998; Zierhut et al. 2000) and the atomic-bomb survivors (Minamoto et al. 2004). The focus of many of these reports was the estimation of the radiation dose that led to a cataract, and the time interval it took to appear. Both clinical research with cancer patients receiving radiotherapy, and laboratory studies with nonhuman models have contributed to our understanding that the opacification of the transparent lens is attributable to damage of the germinative epithelium resulting in a defective differentiation of lens fiber cells (Worgul et al. 1976, 1989). Merriam and Focht (1962) from Columbia University in New York reviewed radiotherapy case histories with known lens exposure levels and observed no opacities following single acute doses of less than ~200 R, with the lens tolerating a higher dose without cataract with increased dose fractionation and overall treatment time. There was a dose-dependent latency in the appearance of the opacity after lens exposure, with higher doses showing cataract sooner. However, these early data had significant variability in the reconstruction of the dose to the

lens. LBNL confirmed the clinical cataract incidence is correlated with the percentage of lens in the radiation field (Meecham et al. 1994).

A comprehensive review of recent studies of radiation cataract by Ainsbury et al. (2009) has concluded that the etiology of cataracts is not fully known, but is likely multifactorial, and that much of the published evidence for radiation cataract is contradictory, but points to little or no dose threshold. Ainsbury et al. (2009) also stated that it is not clear whether a mutational mechanism, or one based on lens cell function, differentiation, cell killing, and/or death is operating as the prime mechanism in radiation-induced cataract.

Basil Worgul from Columbia University published a significant set of conclusions from cataract studies of exposed individuals from the Chernobyl nuclear reactor accident (Worgul et al. 2007). Many of the workers received much lower doses to the lens compared with radiotherapy patients, allowing a more quantitative examination of the low-dose response for cataractogenesis. Linear-quadratic dose-response models yielded mostly linear associations with weak evidence for upward curvature. The data did not support the ICRP Publication 60 (ICRP 1991) risk guideline of a 5 Gy threshold for “detectable opacities” from protracted, primarily low-LET, radiation exposures, but rather pointed to a lower dose-effect threshold of <1 Gy. Thus, given that cataract is the dose-limiting ocular pathology in current eye risk guidelines, Worgul et al. (2007) recommended revision of the allowable exposure of the human visual system to ionizing radiation should be considered.

PARTICLE-RADIATION-INDUCED CATARACT

Cucinotta et al. (2001) was the first to report a radiation-linked health detriment among astronauts exposed to galactic cosmic rays and solar particle events during space travel. Participating members of the astronaut corps with experience in space were divided into a “low-dose” group receiving an average exposure of 3.6 mSv, or a “high-dose” group averaging 45 mSv. Comparisons were made with two control groups, one ground-based with only normal commercial flight experience, and a second group comprised of high-altitude military pilots with no space flight experience. Kaplan-Meier analyses of the proportion of surviving participants without cataract revealed correlations between lens opacity and cataract and radiation dose. Both space radiation exposure groups demonstrated evidence of increased lens opacity and cataract, with the effect more prevalent in the high-dose group. Relative hazard ratios at 60 y of age comparing the high-dose group to the low-dose group from space radiation only, indicated significant differences for all cataract types, including nontrace cataract, compared to lens dose from all radiation sources. The authors considered the findings preliminary because of the use of subjective scoring methods.

Chylack et al. (2009) followed up on this observation with a NASA Study of Cataract in Astronauts cross-sectional study of the relationship of exposure to space radiation and the risk of lens opacity. Cross-sectional data for astronauts and matched ground control subjects were analyzed by fitting customized non-normal regression models to examine the effect of space radiation on nuclear, cortical, and PSC opacities. It was determined that galactic cosmic ray exposure may be linked to increased PSC area and the number of PSC centers. No association was found between space radiation and nuclear cataracts. Cross-sectional analysis revealed a small deleterious effect of space radiation for cortical cataract and possibly for PSC cataracts. These results suggested increased cataract risks at smaller radiation doses than have been reported previously.

STUDY OF THE DOSE-DEPENDENT LATENCY FOR CATARACT

It is clear that radiation can cause cataract, and that there is a dose-dependent latency after radiation exposure before cataract appears, and that at low doses the latency is longer

(Medvedovsky et al. 1994). It has been assumed that not much happens during this latency period except some morphological changes to the lens epithelial and fiber cells. LBNL decided that biologically that did not seem plausible. LBNL set out to study the molecular antecedents to frank particle-induced cataract during the latency period to identify molecular markers early enough to allow biological countermeasures to be devised. LBNL proposed to test a number of hypotheses for the mechanism of radiation cataractogenesis. First, that radiation could lead to an increased genotoxic load of damage leading to cataract through a number of intermediate steps leading to altered gene expression. Secondly, that gene expression could be altered due to epigenetic effects. Thirdly, that the effect could be directly on protein expression. And finally, that there was the possibility that these three hypotheses were not mutually exclusive, and that some combination of these alternative mechanisms could be involved.

Prior to addressing these hypotheses in the laboratory, however, several technical approaches needed to be addressed and new methods and experimental models developed. With support of the National Eye Institute, LBNL devised a method to grow nontransformed human lens epithelial (HLE) cells from an anonymous donated fetal source (Blakely et al. 2000) on a supporting extracellular matrix derived from bovine corneal epithelial cells. These karyotypically normal HLE cells grow and proliferate on this substrate supplemented with growth media, with a population doubling time of ~24 h. The growth kinetics of our nontransformed HLE cells was comparable to the growth kinetics of virally-transformed HLE B-3 cells (Andley et al. 1994). However, the two cell types appeared to be quite different morphologically (Blakely et al. 2000). The nonimmortalized HLE cells at high density near confluence begin to differentiate into human lens fiber (HLF) cells, they align with each other in parallel, their actin filaments show bundling, and their nuclei begin to elongate into elliptical shapes and form lentoid bodies as occurs in vivo. In contrast, the transformed HLE B-3 cells do not show any of these indicators of early differentiation.

IN VITRO MOLECULAR RESPONSES OF HLE DURING POST-EXPOSURE LATENCY

LBNL spent several years learning that the post-exposure latency period after exposure of HLE or HLF cells in vitro, or of lens cells of rodents in vivo, is characterized by very active molecular activity, including the expression of a number of key genes and proteins that can persist in vivo after a single low dose of radiation. Several of these studies are summarized below.

Fibroblast Growth Factor-2 (*FGF-2*)

LBNL investigated a role for radiation-induced changes in the expression of basic Fibroblast Growth Factor (*FGF-2*) gene expression as part of the mechanism(s) underlying lens cell injury associated with cataract (Chang et al. 2000). HLE cells were exposed to a single dose of protons ($\text{LET} = 1.18 \text{ keV } \mu\text{m}^{-1}$) or helium ions ($\text{LET} = 7.2 \text{ keV } \mu\text{m}^{-1}$) at the 88-inch cyclotron at LBNL. LBNL showed evidence for cyclical upregulation of *FGF-2* transcription with the first wave of activity that increases five- and eightfold above baseline occurring as early as 0.5 h after exposure to proton or helium ion radiation, followed by another wave of increased transcription at 2–3 h post-radiation (Fig. 5). Immunofluorescence results confirm the enhanced prevalence of *FGF-2* protein in a rapid period after exposure to particle radiation, followed by another wave of increased activity at 6 h post-irradiation. Total *FGF-2* protein analysis after radiation exposures shows induced expression of three *FGF-2* isoforms, with an up to twofold increase in the 18 kDa low molecular weight species. LBNL postulated that dysfunctional regulation of *FGF-2* expression might cause down regulation of the apoptotic mechanism, resulting in the

migration and differentiation of damaged lens cells leading to aberrant lens crystallin expression and lens opacification.

Cyclin-Dependent Kinase Inhibitors (CDKIs)

Radiobiologists have documented that the damage sensor genes *ATM* and *ATR* are activated by exposure to ionizing radiations [for review, see Chen et al. (2011)]. There is a cascade of signal transducers and effectors downstream of these genes that are crucial to a number of important cell functions. Among these important control mechanisms are three gene families that regulate the transition from G1 phase to S phase that is pivotal to differentiation. Cells must stop dividing to differentiate. The *CIP/KIP* family of *p21*, *p27*, and *p57* is one of the cyclin-dependent kinase inhibitor (CDKI) gene families controlling that transition. The other two are the *INK4* and the *pRB* gene families. Most biologists recognize a critical role for *p21* (also known as *CDKN1A* or *p21^{CIP1/WAF1}*) in cell cycle kinetics, DNA synthesis, and stress responses leading to cell death by apoptosis. A lesser-known function of *CDKN1A* is linked to stem cell differentiation in a number of cell lineages (Dotto 2000). In our culture system, both transcription and translation of *p21^{CIP1/WAF1}* and *p57^{KIP2}* increased as HLE cells differentiate into fiber cells in culture.

Chang et al. (2005) measured *CDKN1A* gene and protein expression in HLE cells in a time-course before and after a single 4 Gy radiation dose of one of three different types of radiation, x rays, protons, or high-energy iron ions. LBNL quantitatively demonstrated that transcription and translation of *CDKN1A* are both temporally regulated after radiation exposure, and that qualitative differences exist in the distribution of CDKN1A immunofluorescence signals after exposure to each of these three radiations suggesting that LET effects play a role in the misregulation of gene function in these cells (Figs. 6 and 7).

Cell Adhesion Molecules

Cell adhesion molecules (CAMs) are proteins that anchor cells to each other and to the extracellular matrix (ECM), but whose functions also include signal transduction, differentiation, and apoptosis. LBNL postulates that particle radiations modulate CAM expression and this contributes to radiation-induced lens opacification. LBNL observed dose-dependent changes in the expression of beta 1-integrin and *ICAM-1* in exponentially growing and confluent cells of our differentiating human lens epithelial cell model after exposure to particle beams. Using monoclonal antibodies that are specific to each CAM, LBNL monitored the intracellular localization of beta 1-integrin and *ICAM-1* and showed that both exponentially-growing and confluent, differentiating cells demonstrated a dramatic proton-dose-dependent modulation (up regulation for exponential cells, down regulation for confluent cells) and a change in the intracellular distribution of the beta 1-integrin, compared to unirradiated controls (Fig. 8). In contrast, there was a dose-dependent increase in *ICAM-1* immunofluorescence in confluent, but not exponentially growing cells. These results suggest that proton irradiation down regulates beta 1-integrin and up regulates *ICAM-1*, potentially contributing to cell death or to aberrant differentiation via modulation of anchorage and/or signal transduction functions (McNamara et al. 2001).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that act as key regulators of tissue remodeling (van Kempen and Coussens 2002). Endogenous inhibitors called tissue inhibitors of metalloproteinases (TIMPs) often control the functional effects of these enzymes. TIMPs have been studied in adult post mortem human lens, in post-cataract lens capsular bags, and in immortalized human lens exposed to cytokines and other stressors. MMPs have been studied in rat lens epithelial and fiber cells and TIMPs in whole lenses (Tamiya et al. 2000; Sachdev et al. 2004). Significant differences exist in the expression patterns with these various models. To characterize expression of MMPs, their

physiological inhibitors (TIMPs) and other lens fiber cell differentiation in HLE cells, LBNL quantitatively analyzed the kinetics of expression of 12 MMPs. LBNL demonstrated cell stage-specific expression of MMP family of genes during lens fiber differentiation, and radiation-induced alterations in the misregulation of MMP expression after proton exposure (Fig. 9). Our data indicate that radiation exposure may lead to differences in the expression of radiation stress responses, which may impact selective ECM remodeling and cell differentiation (Chang et al. 2007).

Cell Communication Channels

To understand the molecular basis of radiation-induced cataract, it is important to identify those genes with altered expression or function due to radiation exposure. Regulation of cell differentiation is dependent on the interplay among integrins, extracellular matrix ligands and cytoskeletal elements. In vivo, the expression and distribution of adhesion molecules in lens cells are dependent on the differentiation status of the cells. LBNL have evidence showing that in vitro models of human lens cell differentiation confirms the in vivo switch in $\alpha 6$ -integrin isoform predominance from $\alpha 6B$ to $\alpha 6A$ in the transition from epithelial to fiber cell-type. Radiation exposure of differentiating fiber cells decreases the $\beta 1$ -integrin-protein expression, and increases $\alpha 6B$ -integrin expression, leading to a subsequent reversion to lens epithelial-like ratios with $\alpha 6B$ dominance. These radiation-induced transient changes in integrins may be crucial to mechanisms of radiation damage leading to cataract.

Connexins are molecular aggregates of intercellular channels between cells composed of a pair of hexameric assemblies of integral membrane proteins belonging to the connexin (Cx) family. Three connexins are expressed in the mammalian lens: Cx43 proteins are reported to be predominant in epithelial cells, while Cx46 and Cx50 proteins are more prevalent in fiber cells. Quantitative comparison of the level of transcription in exponentially-growing HLE cells show that these cells are more transcriptionally active than the HLF cells in that there are >20 times more Cx50 transcripts normally expressed in the HLE cells than in the differentiating HLF cells. A single dose of radiation can further induce upregulation of Cx50 transcription in the HLE cells. A single dose of radiation can further induce upregulation in the HLF cells. The level of induction is LET dependent with ~15-fold at 3 h after 1 Gy iron ions, and >30-fold at 5 h after proton exposure when normalized against the unirradiated control samples.

Immunofluorescence results show that Cx50 protein is transiently upregulated at 5 h after 4 Gy iron ions exposure and Western analysis shows Cx50 protein is overexpressed up to threefold above controls at 4 h after 4 Gy of iron exposure. These results support our hypothesis that particle radiation alters the molecular integrity of intracellular channel proteins and impacts intracellular communications.

IN VIVO/EX VIVO PARTICLE CATARACTS

In vivo confirmation of our in vitro HLE work was essential to make the link to frank cataracts not possible to study in vitro. Sprague-Dawley rats were irradiated in a pilot study at the NASA Space Radiation Laboratory located at Brookhaven National Laboratory using high-energy iron or titanium ions, since these particle beams are representative of high-LET particles encountered in space travel. Doses of either 0, 10, or 100 cGy were studied. The goal was to complete immunohistochemistry of early marker proteins on nascent particle-induced Sprague-Dawley cataracts to correlate with molecular changes in human lens data in vitro.

The lenses of these animals were examined by a veterinarian ophthalmologist for cataract scoring with a slit-lamp examination while the pupils were fully dilated at monthly intervals

over a period of 9 mo after exposure. At the time of terminal necropsy, photographic images of the lenses were obtained ex vivo. The harvested lenses were either frozen without fixation for mRNA and protein analyses, or fixed with paraformaldehyde for immunohistochemical analyses with specific probes.

Ex vivo images of lenses from unirradiated control animals at 9 mo after exposure showed indications of aging, but were primarily clear without opacifications. In contrast, the lenses of animals receiving 100 cGy revealed anterior cortical cataracts. Densitometric analyses of the opacification of the lenses of these animals were analyzed using the National Institutes of Health Image J program to quantitate relative differences in light units along the axis of the analysis between control and irradiated lenses. The data clearly showed light scattering elements. Detailed analysis of ex vivo images of lenses from 100 cGy irradiated animals at 9 mo after 600 MeV amu^{-1} iron ions demonstrated PSC dots of opacification. In vivo imaging from other lenses from the same dose group revealed spokes and annular rings.

IN VIVO MOLECULAR RESPONSES

Quantitative changes in the profile of extracellular matrix and adhesion molecules in rats that were exposed to either 10 or 100 cGy of the same iron ion beam showed that a single low dose of 600 MeV amu^{-1} iron induces a significant three- to fourfold increase in genes in CAM and ECM functional groups in lenses with early cataractous changes 9 mo after exposure. 100 cGy iron-ion-irradiated rat lenses show a completely different gene response. Only two genes in the ECM and CAM series were increased two- to threefold in cataractous lenses, and two CAM series genes were significantly down-regulated two- to threefold 9 mo after exposure. Low-dose irradiated lenses showed surprisingly high, persistent transcriptional change in the expression of many genes including, $\beta 1$ -integrin, *MMP-2*, *MMP-9*, *TGF- $\alpha 1$* , integrin $\alpha 4$, and integrin $\alpha 5$. Of particular interest is the greater than threefold change in the CD44 antigen in these lenses. These observations are consistent with the reports of CD44 involvement cataractous human lenses (Nishi et al. 1997; Saika et al. 1998; Mokbel et al. 2010).

The high responsiveness of *ECM* gene families demonstrated after a low radiation dose to the lens in vivo contrasts with the decreased gene expression seen after a 10-fold higher radiation dose. This suggests different kinetics in the responses, or a different set of responding gene networks. These data also suggest significant dose-dependent differences exist in the response of the lens to particle radiation damage with high doses dampening the low-dose stress responses prior to the onset of cataractous changes.

EMERGING MOLECULAR BIOLOGY OF PARTICLE-RADIATION MECHANISMS RELEVANT TO RADIOTHERAPY

Several new areas of particle radiation research have applied discoveries generated during research in the laboratory to the development of clinical studies. This translational research can confirm relevant mechanisms of action. The importance of understanding the functional relevance of gene alterations in human cancers has become clear (Chang et al. 2009). Gene expression signatures represent units of underlying biological activity linked to known biochemical pathway structures. These constitute a framework to study the processes by which information propagates through cellular networks, and elucidates the relationships of fundamental modules to cellular and clinical phenotypes. A key strength of this approach is that it can uncover signatures of pathway-related activities driven by unknown molecular mechanisms with unbiased and automated statistical analyses (Chang et al. 2009).

Human malignant melanoma is a very radioresistant tumor. Gene expression profiles of six melanoma cell lines exposed to 2 Gy of either 200 kVp x rays or 290 MeV amu⁻¹ carbon ions indicated that all of the melanoma lines were more radiosensitive to carbon ions compared with x rays, and that 22 genes, including nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKB1A) responded to carbon ions in all six cell lines, based on analysis of variance (ANOVA) filtering ($p < 0.001$) (Matsumoto et al. 2008). In four cell lines, 173 genes responded in common to carbon ions. Many down-regulated genes including the cell cycle-related genes were more responsive to carbon than x rays. In contrast, most of the up-regulated genes including the *p53* target genes responded to both carbon ions and x rays. The findings suggest that down-regulation of gene expression plays a key role in the response to carbon ions, and that regulation of cell cycle-related genes and induction of prolonged G2/M arrest may be responsible for the extra sensitivity to carbon ions, whereas *p53*-related genes may have similar roles in the sensitivities to both carbon ions and x rays.

Similar studies comparing carbon ions and x rays have been completed investigating different mechanisms of cell death in radiosensitive and radioresistant *p53* mutated head and neck squamous cell carcinoma (HNSCC) cell lines (Maalouf et al. 2009). High-LET carbon ion irradiation induced distinct types of cell death in HNSCC cell lines and showed an increased effectiveness compared with x rays. However, one very radioresistant *p53*-mutated HNSCC cell line (SQ20B) repopulated. The authors indicate that this may explain the potential locoregional recurrence sometimes observed among some HNSCC patients treated with hadron therapy (e.g., protons or ¹²C ions), and this may require adjuvant therapy.

Glioblastoma multiforme is one of the most difficult human brain tumors to treat successfully. Studies of the mechanisms of heavy ion-induced *p53*-independent glioma cell death induced by carbon ion irradiation was recently reported to be regulated through a MEK-ERK-mitochondria-caspase cascade that culminates in apoptotic death, and may be key to improvement in the clinical outcome of glioma therapy by carbon-ion radiation (Tamiya et al. 2000). MEK inhibitors or overexpression of a dominant-negative ERK failed to significantly inhibit x-ray-induced cell death in the glioma lines tested.

Tumor metastases remain a major problem in eradicating cancer from a patient. Sublethal photon irradiation was recently suspected to increase tumor cell motility and invasiveness of human malignant glioma cells (Wild-Bode et al. 2001). This previously unknown biological effect of irradiation is *p53* independent, involves enhanced $\alpha_v\beta_3$ integrin expression, an altered profile of matrix metalloproteinase-2 and matrix metalloproteinase-9 (*MMP-2* and *MMP-9*) expression and activity, altered membrane type 1 *MMP* and tissue inhibitor of metalloproteinases-2 expression, and an altered *BCL-2/BAX* rheostat favoring resistance to apoptosis. *BCL-2* gene transfer and irradiation cooperate to enhance migration and invasiveness in a synergistic manner.

The metastatic capabilities of several malignant tumor cells after irradiation with photon, proton, and carbon ion beams have been compared (Ogata et al. 2005). The biological properties of highly aggressive HT1080 human fibrosarcoma cells were examined to assess their metastatic processes in terms of cell adhesion capability to extracellular matrix, expression of integrins, cell migration, cell invasive capability, and matrix metalloproteinase-2 activity in vitro. The metastatic capabilities of LM8 mouse osteosarcoma irradiated with carbon ion or photon beam in the syngeneic mice were investigated. Both proton and carbon ion irradiation decreased cell migration and invasion in a dose-dependent manner and strongly inhibited matrix metalloproteinase-2 activity. In contrast, lower x-ray irradiation promoted cell migration and invasion concomitant with up-

regulation of $\alpha_v\beta_3$ integrin. For cancer cells treated with carbon-ion irradiation, the number of pulmonary metastasis was decreased significantly in vivo. These findings suggest that particle irradiation suppresses metastatic potential even at lower dose, whereas photon irradiation promotes cell migration and invasive capabilities at lower dose level, and provide preclinical evidence that ion beam radiotherapy may be superior to conventional photon beam therapy in possible preventive effects on metastases of irradiated malignant tumor cells.

The effect of photon- and carbon-ion irradiation on glioma cell migration has been studied with U87 and Ln229 glioma cells (Rieken et al. 2011). Migration was analyzed 24 h after irradiation. Fluorescence-activated cell sorting analysis was performed in order to quantify surface expression of integrins. Single photon doses of 2 and 10 Gy enhanced $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin expression and caused tumor cell hypermigration on both vitronectin and fibronectin. Compared to integrin expression in unirradiated cells, carbon ion irradiation caused decreased integrin expression and inhibited cell migration on both vitronectin and fibronectin. This provides evidence that photon radiotherapy enhances the risk of tumor cell migration and subsequently promotes locoregional spread via photon induction of integrin expression. In contrast to photon RT, carbon ion RT causes decreased integrin expression and suppresses glioma cell migration on both vitronectin and fibronectin, thus promising improved local control.

The effects of 4 MV x ray and 290 MeV amu^{-1} carbon ions on nonsmall-cell lung cancer cell aggressiveness and gene expression have been compared using A549 (lung adenocarcinoma) and EBC-1 (lung squamous cell carcinoma) cell lines (Akino et al. 2009). Proliferative, migratory, and invasive activities by cell proliferation assay, Boyden chamber assay, and Matrigel chemoinvasion assay, respectively. cDNA microarray and reverse transcription polymerase chain reaction were also performed to assess mRNA expression alteration. The authors report that X-irradiation increased cell proliferation of A549 cells at 0.5 Gy, whereas high-dose x-ray reduced migration and invasion of A549 cells. By contrast, carbon beam irradiation did not enhance proliferation, and it reduced the migration and invasion capabilities of both A549 and EBC-1 cells more effectively than did X-irradiation. Carbon beam irradiation induced alteration of various gene expression profiles differently from x-ray irradiation. mRNA expression of *ANLN*, a homologue of anillin, was suppressed to 60% levels of basal expression in carbon-beam irradiated A549 cells after 12 h. Carbon beam effectively suppresses the metastatic potential of A549 and EBC-1 cells. Carbon beam also has different effects on gene expressions, and downregulation of *ANLN* was induced only by carbon beam irradiation.

Carbon ion irradiation inhibits the expression of the anillin (*ANLN*) gene, which is regulated by the activation of the phosphatidylinositol-3-kinase (*PI3K*)/*Akt* signaling pathway associated with metastasis (Suzuki et al. 2005). Recent investigations comparing the effects of carbon ion irradiation on the *PI3K*/*Akt* signaling pathway to those of photon irradiation showed that carbon ion irradiation of human lung adenocarcinoma cells A549 decreased invasion more effectively than photon irradiation (Ogata et al. 2011). The carbon ion irradiation reduced the nuclear localization of *ANLN* at lower dose, but did not affect its expression. Low-dose carbon ion irradiation also reduced the level of phosphorylated Akt compared to untreated controls, whereas photon irradiation did not. These results suggested that carbon-ion irradiation effectively suppresses the metastatic potential of A549 cells by suppressing the *PI3K*/*Akt* signaling pathway.

Pancreatic cancer is an aggressive disease that responds poorly to conventional photon radiotherapy. Carbon-ion irradiation suppressed the migration of several human pancreatic cell lines (MIAPaCa-2, BxPC-3, and AsPC-1); diminished the invasiveness of MIAPaCa-2;

and tended to reduce the invasion of BxPC-3 and AsPC-1 (Fujita et al. 2012). However, carbon-ion irradiation increased the invasiveness of PANC-1 through the activation of plasmin and urokinase-type plasminogen activator. This is the first study showing that carbon-ion irradiation induces the invasive potential of a tumor cell line. Administration of serine protease inhibitor (SerPI) alone failed to reduce carbon-ion-induced PANC-1 invasiveness, whereas the combination of SerPI and Rho-associated coiled-coil forming protein kinase (ROCK) inhibitor suppressed it. PANC-1 also showed mesenchymal-amoeboid transition when treated with SerPI alone. Further in vivo studies are required to examine the therapeutic effectiveness of radiotherapy combined with inhibitors of both mesenchymal and amoeboid modes of tumor cell motility.

Complex chromosomal exchanges are responsible for the increased effectiveness of carbon ions compared to x rays at the first post-irradiation mitosis (Lee et al. 2010). Cytogenetic changes have been investigated in the progeny of two normal human fibroblast cell strains after exposure to x rays or densely ionizing 9.8 MeV amu⁻¹ carbon ion irradiation (Zahnreich et al. 2010). The cells were regularly subcultured up to senescence. The transition to senescence was determined by measurement of population doubling numbers and senescence associated β -galactosidase activity. Chromosomal changes (structural aberrations, tetraploidy) were investigated by solid staining. In temporal proximity to senescence, Zahnreich et al (Zahnreich et al. 2010) noted that there was an increase in the fraction of cells with structural and numerical aberrations for all populations of the two fibroblasts cell strains. The observed changes in the yield of structural chromosomal aberrations were similar for the progeny of controls and irradiated cells, except that a previous irradiation with a high, fractionated x-ray dose resulted in a stronger increase. A significant observation was that delayed tetraploidy in the descendants of cells irradiated with either x rays or carbon ions, regardless of dose, exceeded the level in control cells. An enhanced formation of tetraploid cells occurs concomitantly with senescence, no matter when the cells undergo this transition. However, the time of the onset of senescence depends on previous exposure to radiation. The occurrence of tetraploidy is associated with senescence independently of exposure to radiation.

Macrophages are potent elicitors of inflammatory reactions that can play both positive and negative roles in radiotherapy. Comparisons have been made of the effects of 250 kV x rays with those of 9.8 MeV amu⁻¹ carbon ions on RAW 264.7 macrophages over a wide range of radiation (Conrad et al. 2009). Macrophage functions including vitality, phagocytic activity, production of the proinflammatory cytokines IL-1 β and TNF α and production of nitric oxide were measured. In comparison to lymphocytes and fibroblasts, macrophages showed only a small decrease in vitality after irradiation with either x rays or carbon ions. Proinflammatory cytokines and nitric oxide were induced in macrophages by LPS but not by irradiation alone. X rays or carbon ions had little modulating effect on LPS-induced TNF α production. However, LPS-induced nitric oxide increased in a dose dependent manner up to sixfold after carbon ion irradiation, while x-ray irradiation did not have this effect. Carbon ion irradiation mediated a concomitant decrease in IL-1 β production. Carbon ions also had a greater effect than x rays in enhancing the phagocytic activity of macrophages. These results underscore the greater potential of carbon-ion irradiation with regard to radiobiological effectiveness.

FUTURE RESEARCH NEEDED

This review of experimental evidence comparing low- and high-LET radiation effects on cultured human lens epithelial cells and fibroblasts has profiled pathways deregulated by particle radiation exposure, and described in vivo studies in rats and mice that are in progress to confirm the anatomical development of cataract and the role of specific gene

products in cataract development. New information has been obtained about normal and irradiated lens differentiation mechanisms.

Fig. 10 presents a summary overview of our in vitro and in vivo experimental approach to test the hypothesis that early molecular markers of radiation cataractogenesis will lead to underlying mechanisms and countermeasures. The latency period following lens irradiations and before the appearance of frank opacifications is not a quiescent time at the molecular level. LBNL have demonstrated that several gene families are affected including specific cytokines, cell cycle regulatory genes, and a number of genes that control actin dynamics, cell migration, integrins, adhesion complex remodeling, and cell communication channels. All of these genes appear to impact the normal differentiation of lens epithelial cells into fiber cells that leads to the orchestration of a uniform set of morphological formations (such as evidenced by the incomplete denucleation that occurs at terminal differentiation), and may also impact potential modes of cell senescence or death. Interference with the normal sequence of events may well lead to skewed microanatomy that decreases the clarity of the crystalline lens. Quantitative measurements of dose- and LET-dependent changes in specific proteins may lead to the development of biologically-based countermeasures that may one day prevent radiation-induced cataract.

The example of particle effects on lens cells is a microcosm of some of the topics addressed in the program presented at the 2011 NCRP Annual Meeting focused on scientific and policy challenges of particle radiations in medical therapy and space missions. Key among these topics that address the question of what makes particle radiation so effective were descriptions of the unique physical interactions of charged particles with absorbing materials that have been modeled by theoretical physicists to reveal track structures dependent on particle atomic number and charge. Damage complexity and clustering and the production of short DNA fragments are revealed in biophysical modeling with molecular studies in the laboratory that also show slower DNA repair and evidence of misrepair and enhanced genomic instabilities at high-LET. In addition to quantitative differences in enhanced gene expression after high-LET radiation exposure, qualitative LET-dependent differences in gene expression have been revealed. Non-DNA-based mechanisms are also prevalent from microenvironmental changes in the stroma of high-LET-irradiated tissues. The integration of these underlying mechanisms into biophysical modeling for implementation of particle therapy was also reviewed.

Several unknown factors exist in assessing light- and heavy-ion damage and their importance to relevant outcomes. For example, the consequences of persistent chromosomal rearrangements found in white blood cells from astronauts or cosmonauts after their return to earth are unknown (George et al. 2010) as are the possible role in inducing genomic instability many cell generations after high-LET radiation exposure (Ducray and Sabatier 1998; NA/NRC 2012). The identification of individual genetic susceptibility might require screening of sensitive individuals for certain kinds of radiation work (NCRP 2010). The effect of exposure to low particle fluences and rates on late-occurring tissue effects and the subsequent induction of remodeling of basement membranes in the stroma and extracellular matrix of tissues needs further study. The importance of the enhanced sensitivity of neural behavior following exposure to low doses of particles, as well as the evaluation of the combined effects of multiple stressors, such as particle exposure combined with changes in microgravity or oxygen levels will be crucial.

Why is it important to identify molecular pathways of action for particle effects? LBNL now have the tools to understand the molecular pathology of cancer, and how to use this information to treat individual cancers (Riedel et al. 2008; Harris and McCormick 2010). Unique gene expression pathways are being reported in the literature for human tumors

irradiated with radiations of different qualities (Higo et al. 2006; Hamada et al. 2008; Maalouf et al. 2009). It is a new era for charged particle radiobiology. The human genome has been mapped and is being mined for tumor and normal tissue data on radiation responses. Powerful new genomic and proteomic tools are available that allow us to focus on future individualized medicine. Networks of gene and protein pathways have been identified that show expression profiles change in a dose- and time-dependent fashion after exposure to particles of variable LET. Modern physics and treatment planning is using tailored three-dimensional image guided and intensity modulated scanned particle beam delivery for cancer therapy. Theoretical biophysical modeling is guiding treatment optimization, but more work is needed to understand microdosimetric energy deposition events in individual tissues.

Based on the weight of published radiation effects (i.e., epidemiological evidence with more data in the low-dose range with improved methods of scoring and with longer follow-up) ICRP released a statement on April 21, 2011 on tissue reactions that included new radiation dose limits for tissues including the eye (ICRP 2012). This statement asserted that published data suggest some tissue reaction effects with late manifestation may have lower threshold doses than previously considered. ICRP notes that there is no direct evidence that a single damaged progenitor lens epithelial cell can produce a cataract (the hallmark characteristic of a stochastic effect), and hence radiation-induced lens cataract is still considered a tissue reaction (deterministic effect) with a dose threshold, albeit small (ICRP 2011). However, genetics and age at exposure remain important contributors to outcome. ICRP now recommends an equivalent absorbed dose limit of 0.5 Gy in a single exposure to the lens of the eye. For chronic occupational exposures, ICRP recommends an equivalent dose limit for the lens of the eye of 20 mSv in a year, averaged over defined periods of 5 y, with no single year exceeding 50 mSv. This is one-third of the prior recommended dose limit of 150 mSv y^{-1} (ICRP 2007).

Although uncertainties remain, ICRP recommended that medical practitioners should be made aware that the absorbed dose threshold for circulatory disease might also be as low as 0.5 Gy to the heart or brain. ICRP continues to recommend that optimization of protection be applied in all exposure situations and for all categories of exposure, not only for the whole body, but also for exposures to specific tissues, particularly the lens of the eye, the heart and the cerebrovascular system. Concerns have been published regarding how this new recommendation will be implemented (Englefield 2011; Martin 2011; Rehani et al. 2011).

Despite the progress made to date, much more remains to be learned about the pathways regulating particle-induced effects on cell and tissue homeostasis. With multiple growth factors signaling pathways and their intermediates capable of influencing cell functions, it will be important to determine how all these signaling pathways are coordinated in each tissue type. Genetic approaches to pathway analysis have the potential to provide insight into this complex crosstalk of signaling pathways. More basic research is definitely required to elucidate unknown underlying mechanisms with the perspective of individual genetics and endogenous stress responses.

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Fig. 1. Cross-sectional dependence of cell inactivation, cell transformation and mutation induction as a function of LET (Blakely 1992). The left panel (Tobias and Todd 1967) are inactivation cross section plots for a variety of biological test objects, including human T-1 fibroblasts as a function of LET. The panel on the right (Goodhead and Nikjoo 1987) are cross-sections for various mammalian cell endpoints as a function of LET.

Fig. 2.

Plot of the LET dependence of the aerobic RBE-10 values (relative to x rays) for human AT-2SF and T-1 cells. The RBE values for resistant T-1 fibroblasts are greater than for the radiosensitive ataxia telangiectasia AT-2SF fibroblasts and bracket the range of RBE values reported for a large number of different cell lines (Tobias et al. 1984).

Fig. 3. Schematic depicting a cross-section through a human eye (from Wikipedia) with an enlarged detailed cross-section of the crystalline lens (modified from Google Images).

Fig. 4. Kaplan-Meier survival curves of cataract as a function of time after therapy, with the patient population divided into quartiles according to anterior segment radiation exposure (Meecham et al. 1994).

Fig. 5. *FGF-2* expression in HLE cells after proton irradiation. **Panel A:** shows that cyclical time course of the relative induction of *FGF-2* transcripts in exponentially growing HLE cells irradiated with 4 Gy of proton radiation. Data from several separate experiments are represented in different symbols. **Panel B:** shows enhanced *FGF-2* immunoreactivity at 1.5 h after irradiation, followed by a decrease in fluorescence signals at 3 h post irradiation (Chang et al. 2000).

Fig. 6.

Panel A: CDKN1A immunofluorescence signals in human lens epithelial cells 6 h after exposure to 4 Gy iron ions, protons or x rays. The panels on the left show representative photomicrographs illustrating sporadic CDKN1A expression in the control unirradiated cells. Photomicrographs on the right show radiation-induced up-regulation of CDKN1A (in pink) after exposure to iron ions, protons or x rays. Cells were counterstained with DAPI to show blue nuclei. **Panel B:** Quantitative analysis of CDKN1A (p21) fluorescence intensity after irradiation. Fluorescence intensities of >50 cells were collected for each treatment condition. A high percentage of CDKN1A-positive cells in the respective control samples (gray bars) show relatively low fluorescence levels. The percentage of CDKN1A-positive cells in the 4 Gy irradiated samples (black bars) is shifted toward higher fluorescence intensities (Chang et al. 2005).

Fig. 7. p21 expression in lens epithelial cells after exposure to 50 cGy (left panel) or 400 cGy (right panel) iron ions (Chang et al. 2005).

Fig. 8.

β 1-integrin expression in human lens epithelial cells. In unirradiated lens cells β 1-integrin (red fluorescence) changes during differentiation from single focal adhesion points in epithelial cells to a wider distribution over the whole cell membrane in differentiating confluent cells. Proton irradiation increases β 1-integrin expression, and changes the distribution 6 h after single graded proton doses. Once the cells begin differentiation, the effect is reversed, and β 1-integrin expression and distribution decreases.

Fig. 9. Quantitative evaluation of radiation-induced alterations in MMP-2, -3, -9, and TIMP-1 mRNA expression in exponentially-growing (E) and quiescent (C) cells in a time course after 4 Gy 1 GeV amu^{-1} iron or 55 MeV amu^{-1} proton irradiation (Chang et al. 2007)

Fig. 10. Lens fiber cell differentiation involves several radiation-responsive signal transduction pathways. Hypothesis: Early molecular markers of radiation cataractogenesis will lead us to underlying mechanisms and countermeasures.