Final Report

# Low-Dose Radiation Cataract and Genetic Determinants of Radiosensitivity

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PI: Norman J. Kleiman

Institution: *Department of Environmental Health Sciences*
*Mailman School of Public Health*
*Columbia University 722 West 168th Street, 11th floor*
*New York, NY 10032*

Team members: David J. Brenner, Lubomir B. Smilenov, Eric J. Hall
*The Center for Radiological Research, College of Physicians & Surgeons,*
*Columbia University, New York 10032*

## Executive Summary:

The lens of the eye is one of the most radiosensitive tissues in the body. Ocular ionizing radiation exposure results in characteristic, dose related, progressive lens changes leading to cataract formation. While initial, early stages of lens opacification may not cause visual disability, the severity of such changes progressively increases with dose until vision is impaired and cataract extraction surgery may be required. Because of the transparency of the eye, radiation induced lens changes can easily be followed non-invasively over time. Thus, the lens provides a unique model system in which to study the effects of low dose ionizing radiation exposure in a complex, highly organized tissue. Despite this observation, considerable uncertainties remain surrounding the relationship between dose and risk of developing radiation cataract. For example, a growing number of human epidemiological findings suggest significant risk among various groups of occupationally and accidentally exposed individuals and confidence intervals that include zero dose. Nevertheless, questions remain concerning the relationship between lens opacities, visual disability, clinical cataract, threshold dose and/or the role of genetics in determining radiosensitivity. Experimentally, the response of the rodent eye to radiation is quite similar to that in humans and thus animal studies are well suited to examine the relationship between radiation exposure, genetic determinants of radiosensitivity and cataractogenesis. The current work has expanded our knowledge of the low-dose effects of X-irradiation or high-LET heavy ion exposure on timing and progression of radiation cataract and has provided new information on the genetic, molecular, biochemical and cell biological features which contribute to this pathology. Furthermore, findings have indicated that single and/or multiple haploinsufficiency for various genes involved in DNA repair and cell cycle checkpoint control, such as Atm, Brca1 or Rad9, influence cataract development and thus radiosensitivity. These observations have direct applicability to various human populations including accidentally exposed individuals, interventional medical workers, astronauts and nuclear plant workers.

## Introduction.

In 2012, the ICRP, as well as other advisory groups, released new guidelines and recommendations concerning ocular ionizing radiation exposure. ICRP significantly lowered the presumptive threshold for radiation cataract to 0.5 Gy, regardless of whether exposure was acute, protracted or chronic. Similarly, the occupational lens exposure limit was lowered, from 150 mSv/yr to an average of 20 mSv/yr over 5 years, with no single year exceeding 50 mSv. These new recommendations were based on new human epidemiological evidence concerning ocular risks and are likely to have significant implications for occupational exposure and the need for eye protection, for example in fields such as interventional cardiology. Nevertheless, there is much to be learned concerning which individuals are most at risk and what the underlying pathophysiology is that drives the formation of this particular type of lens opacity.

The lens is considered one of the most radiosensitive tissues in the body and the primary pathology associated with ionizing radiation exposure, cataract, or loss of transparency of the lens, is easily observed *in-vivo*. Thus, the lens provides a unique model system in which to study the effects of low dose ionizing radiation exposure in a complex, highly organized tissue. Considerable uncertainties, however, surround the relationship between dose and cataract development. In addition, despite recent downward revisions in ICRP ocular exposure guidelines in other countries, current NCRP and NRC recommendations continue to reflect significantly higher limits based on decades earlier studies of radiation cataract in limited numbers of subjects. These earlier guidelines were predicated on the view that cataractogenesis is deterministic with a relatively high threshold radiation dose required before the appearance of visually disabling lens. It should be noted that the earlier studies generally had short follow-up periods, failed to take into account increasing latency as dose decreased, did not have sufficient sensitivity to detect early lens changes and had relatively few subjects with doses below a few Gy. Newer data from animal models and from exposed human populations are statistically consistent with a small or even zero dose threshold. These include populations exposed to far lower doses of radiation, including those undergoing CAT scans, radiotherapy, the astronaut pool, atomic bomb survivors, residents of radioactively contaminated buildings, interventional physicians and medical workers and the Chernobyl accident “Liquidators”. In these cases, dose-related lens opacification at significantly lower exposures than the earlier dose limits was reported. Thus, the concept of a dose threshold is critical not only to theories regarding the mechanism(s) of radiation cataract but to actual risk assessment in a variety of exposed populations.

In addition to these exposed cohorts, other epidemiological studies suggest the existence of radiosensitive human sub-populations, which further complicates assessment of a putative threshold radiation cataract dose. For example, our group, and others, have suggested that individuals haplo-insufficient for one or more genes involved in DNA damage repair and/or cell cycle checkpoint control may be especially susceptible to the cataractogenic effects of ionizing radiation. If this hypothesis were true, it would be unethical to put radiosensitive individuals in situations where they might receive relatively high doses. At the same time, inclusion of such individuals in epidemiological studies may distort the shape of the dose-response curve such that a linear extrapolation from high to low doses may be invalid. These observations have

clear implications for radiotherapy, diagnostic procedures and for those occupationally exposed to ionizing radiation, such as interventional medical personnel or the astronaut core and may aid in determining future national terrestrial and space radiation risk policies.

Our research utilizes radiation cataract as a model system to study the effects of low-dose ionizing radiation exposure in a complex, highly differentiated tissue. We believe radiation cataract is a useful model to determine the genetic and individual basis for risk from ionizing radiation with particular relevance to carcinogenesis and radiation induced DNA damage, repair and cell cycle control. These studies are also designed to help elucidate the mechanistic nature of radiation induced biological damage and determine the relative contribution of genetic defects affecting radiosensitivity. The unique morphology of the lens and the ease of non-invasive observation of its radiation response facilitate molecular and mechanistic investigations resulting in unrepaired or misrepaired DNA damage.

Previous funded studies from our laboratories demonstrated that mice haplo-insufficient for *Atm* (that contain one good copy and one bad copy of the *Atm* gene and a correspondingly reduced amount of ATM protein) develop radiation cataracts after exposure to 500 mGy x-ray earlier and with more severity than wild type animals (Fig. 1). This finding lead to speculation that individual differences in time of cataract onset and rate of progression in various human exposed cohorts, for example the astronaut core, Chernobyl Liquidators or interventional physicians, might be explained, in part, by individual genetic differences in radiosensitivity.

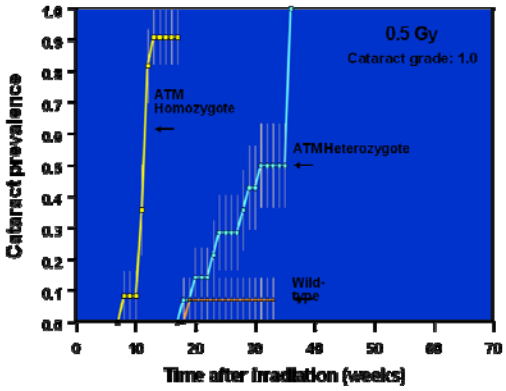


Fig. 1 Time course of cataract onset and progression; radiation cataract stage 1, in *Atm* homozygous and heterozygous animals, as compared to unirradiated controls, following exposure to 0.5 Gy X-ray. *Rad. Environ. Biophys*. **45**, 2006**.**

The research described in this Technical Report seeks to expand the library of genes involved in DNA damage recognition and repair and/or cell cycle checkpoint control beyond *Atm* as well as to examine the putative lens dose threshold for radiation-induced changes and associated histopathological changes, and to investigate the possible relationship(s) between heterozygosity and/or haploinsufficiency for one or more such genes and the time of onset or progression of radiation induced lens changes in various animal models.

**Project Aims and Findings.**

This proposal was based on the hypothesis that, following exposure to low-dose, low-LET x-rays, aberrantly dividing and/or differentiating cells in the pre-equatorial region of the lens epithelium migrate to the posterior pole of the lens where they become opaque lens fiber cells. The plan was to investigate mechanisms of cataractogenesis by observing opacities produced by x-irradiation of mice that were

haplo-insufficient for one or more genes involved in DNA damage recognition and repair and/or cell cycle checkpoint functions. The genes chosen included *Atm*, *Brca1* and *Rad9*, based on experiments with cells *in-vitro* which indicate that heterozygosity for these genes confers radiosensitivity. There are corresponding human homologues for these genes and mutations and/or polymorphisms have been identified in a few percent of the human population. Thus, heterozygosity for these or similar genes could account for the unexpected observation of earlier onset or faster progression of cataracts in some individuals in various exposed cohorts.

Initial findings indicated that animals haploinsufficient, either singly or in combination, for *Atm and Rad9* had faster onset and rate of progression than wild-type controls. Furthermore, animals haploinsufficient for both Atm and Rad9, were more likely to reach stage 2 opacities than singly haploinsufficient mice (Fig 2).

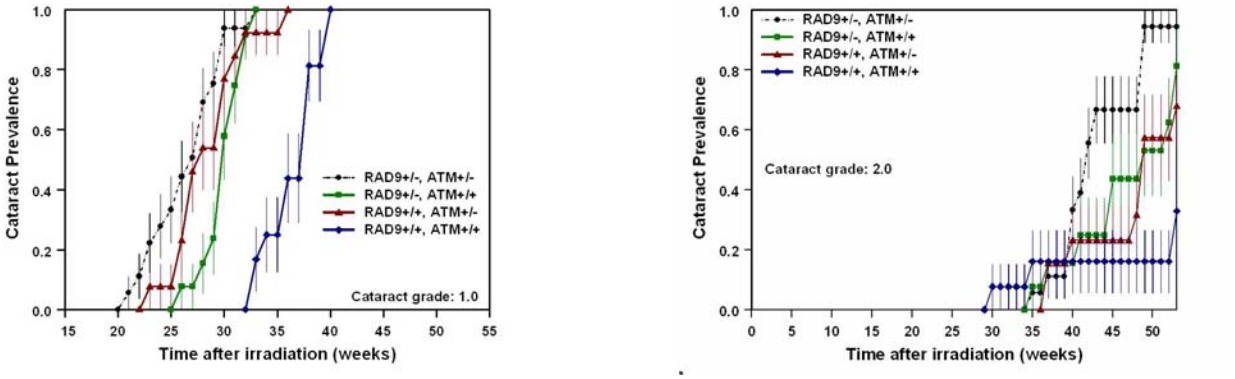


Fig. 2. Cataract prevalence for grade 1 (left panel) or grade 2 opacities (right panel) in mice singly or doubly haploinsufficient for *Atm* or *Rad9*. *Radiat*. *Res* **168**, 2007**.**

Additional work from the laboratory, examining the effect(s) of single or multiple haploinsufficiency for *Atm* and *Brca1,* suggest the relative influence of dual haploinsuffiency on radiation cataract onset and progression may be more complex, as the *Atm/Brca1* double heterozygous animals are no more likely to develop radiation cataract than either *Atm* or *Brca1* single heterozygotes (Fig. 3).

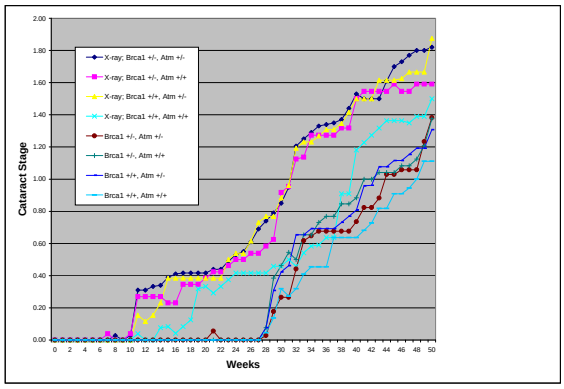


Fig. 3. Influence of dual haploinsuffiency for Atm and Brca1 on radiation cataract development after exposure to 500 mGy X-ray. *Invest. Ophthalmol. Vis. Sci.* **47**, 2006.

Nevertheless, as radiation cataract has been shown to involve, *a priori*, genotoxic damage to the lens epithelium, it is likely that expression of these and other genes which have important roles in recognizing and repairing radiation induced DNA damage, inducing cell cycle arrest and initiating apoptosis, are likely to be closely related to risk of radiation-induced lens changes..

Such animal experimental work has broad implications for human exposure and risk assessment and suggests that individual human genetic differences in radiosensitivity might help explain the wide variation in reported time of cataract onset, degree of opacification and subsequent progression to visual disability among exposed populations. More importantly, these findings have important implications for both radiotherapy and occupational exposure in radiosensitive subsets of the human population. We hypothesize that cellular and molecular pathways of the biological response to ionizing radiation exposure in the lens has fundamental relevance and similarity to that in other tissues and that radiation cataract arises from damaged or misrepaired DNA and subsequent errors in cell cycle control, division and differentiation, similar to that observed in carcinogenesis.

This view is supported by the 2011 report from the ICRP which states *"The precise mechanism of radiation cataractogenesis is not known, but genomic damage resulting in altered cell division, transcription and/or abnormal lens fibre cell differentiation is considered to be the salient injury, … heterozygosity for genes involved in cell cycle checkpoint control, DNA damage recognition, or DNA repair might also contribute to this phenomenon"*.

At the same time as our work with genetic determinants of radiosensitivity, we also hoped to demonstrate that dose-related significant lens changes would occur after as little as 50 mGy X-ray exposure (Fig. 4). Furthermore, we hoped to see whether unirradiated lens cells were affected by irradiated regions and whether unirradiated regions offered some measure of protection to irradiated lens fiber cells (Fig. 5). Lastly, we hypothesized that histological examination of micronuclei and fragmented nuclei frequency in lens epithelium from these animals would support an increased likelihood of apoptotic and clastogenic events in the germinative and transitional regions of the lens epithelium as compared to unexposed controls. These hypotheses were proved accurate (Figs 6A,B; Fig. 7A,B).

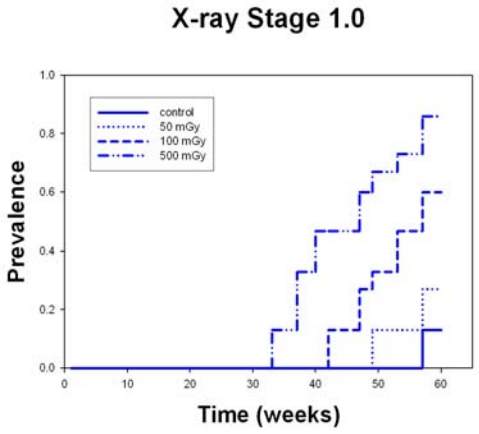


Fig. 4. Dose-dependent radiation-induced lens changes are noted after as little as 50 mGy X-irradiation of the rat lens, well within the normal lifetime of the animal, suggesting that existing theories concerning the deterministic nature of radiation cataract may need to be re-examined.

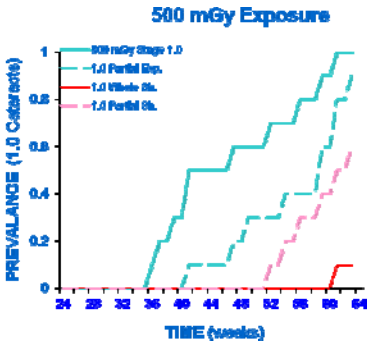


Fig. 5. Influence of neighboring shielded or unshielded portions of the lens on cataract onset and progression in rats exposed to 500 mGy X-ray. The data suggests both a protective "bystander effect" in that the time of onset of lens changes in partially shielded lenses is significantly greater than that of unshielded eyes. At the same time, a negative "bystander effect" on unexposed tissue is suggested in that the onset of lens changes in the shielded portion of partially irradiated lenses is significantly shorter than that in totally shielded eyes.

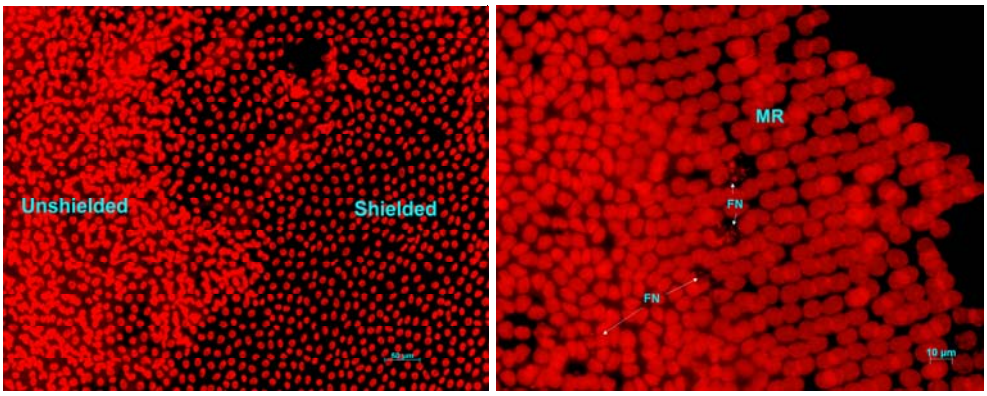


Fig. 6A. (left panel) Disorganization of the germinative zone in partially shielded lenses (propidium iodide nuclear staining). Fig. 6B. (right panel) Fragmented nuclei (FN) in the meridional rows (MR) of irradiated lens epithelium.

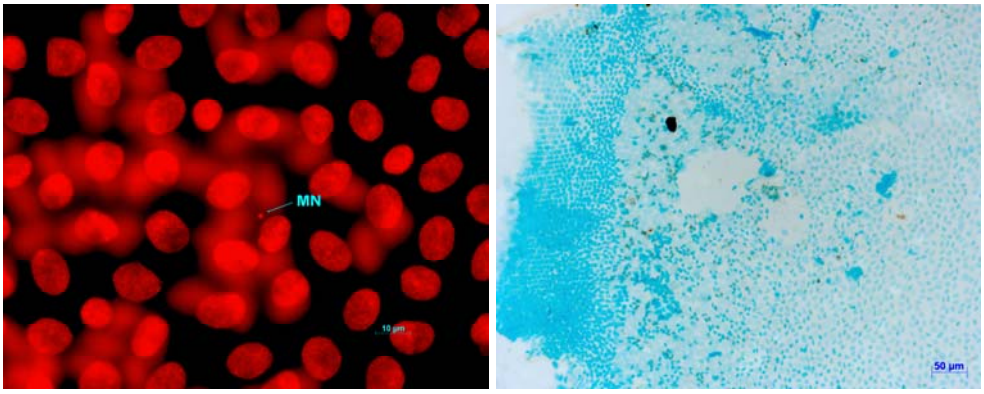


Fig. 7A (left panel) Micronuclei in the dividing population of lens epithelium exposed to 500 mGy X-ray; propidium iodide nuclear staining. Fig. 7B (right panel) Apoptotic cells (stained brown) in a lens epithelial flat mount following *in-vivo* exposure to 500 mGy X-ray.

## Publications:


Kleiman, N.J. David, J**.,** Elliston, C.D., Hopkins, K.M., Smilenov, L.B., Brenner, D.J., Worgul, B.V., Hall, E.J. and Lieberman, H.B. (2007) Mrad9 and Atm haploinsufficiency enhance spontaneous and X-ray-induced cataractogenesis in mice. *Radiat. Res*. **168**, 567-573.

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