Introduction to the Special LDLensRad Focus Issue

Authors: Ainsbury, Elizabeth A., Dalke, Claudia, Mancuso, Mariateresa, Kadhim, Munira, Quinlan, Roy A., et al.

Source: Radiation Research, 197(1) : 1-6

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RADE-21-00188.1

RADIATION RESEARCH **197**, 1–6 (2022)
0033-7587/22 $15.00

DOI: 10.1667/RADE-21-00188.1

COMMENTARY

Introduction to the Special LDLensRad Focus Issue

Elizabeth A. Ainsbury,[a,1,2] Claudia Dalke,[b,2] Mariateresa Mancuso,[c,2] Munira Kadhim[d], Roy A. Quinlan,[e] Tamara Azizova,[f] Lawrence T. Dauer,[g] Joseph R. Dynlacht,[h,2] Rick Tanner[a] and Nobuyuki Hamada[i,2]

[a] *Public Health England (PHE) Centre for Radiation, Chemical and Environmental Hazards, Oxon, United Kingdom;* [b] *Institute of Developmental Genetics, Helmholtz Zentrum München GmbH - German Research Center for Environmental Health, Neuherberg, Germany;* [c] *Laboratory of Biomedical Technologies, Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile (ENEA), Rome, Italy;* [d] *Department of Biological and Medical Sciences, Faculty of Health and Life Sciences, Oxford Brookes University, Oxford, OX30BP, United Kingdom;* [e] *Department of Biosciences, Durham University, Durham DH1 3LE, United Kingdom;* [f] *Southern Urals Biophysics Institute, Ozyorsk, Russia;* [g] *Memorial Sloan Kettering Cancer Center, New York, New York;* [h] *Department of Radiation Oncology, Indiana University School of Medicine, Indianapolis, Indiana; and* [i] *Radiation Safety Unit, Biology and Environmental Chemistry Division, Sustainable System Research Laboratory, Central Research Institute of Electric Power Industry (CRIEPI), Komae, Tokyo, Japan*

Ainsbury EA, Dalke C, Mancuso M, Kadhim M, Quinlan RA, T Azizova T, Dauer LT, Dynlacht JR, Tanner R, Hamada N. Introduction to the Special LDLensRad Focus Issue. Radiat Res. 197, 1–6 (2022).

Recent epidemiological and experimental animal data, as well as reanalyses of data previously accumulated, indicate that the lens of the eye is more radiosensitive than was previously thought. This has resulted in a reduction of the occupational lens dose limit within the European Union countries, Japan and elsewhere. This Commentary introduces the work done by the LDLensRad Consortium contained within this Focus Issue, towards advancement of understanding of the mechanisms of low dose radiation cataract.

Recent epidemiological and experimental animal data, as well as reanalyses of data previously accumulated, indicate that the lens of the eye is more radiosensitive than was previously thought. This has resulted in a reduction of the occupational lens dose limit within the European Union countries, Japan and elsewhere (1). For example, the International Commission on Radiological Protection (ICRP) recommended that the dose limit for workers should be reduced from 150 mSv year^{-1} to 20 mSv year^{-1} averaged over 5 years, with no single year exceeding 50 mSv (2). The National Council on Radiation Protection and Measurements (NCRP), which prefers to use absorbed dose when addressing tissue-specific deterministic effects (also referred to as tissue reactions) recently recommended a reduction in the occupational annual lens limit to 50 mGy and advised that members of the public should not exceed 15 mGy annually (3, 4).

While the substantial reduction in dose limits was chiefly driven by recent epidemiological data, both ICRP and NCRP have concluded that the mechanism(s) of low dose radiation cataract induction are still unclear, and that it is currently not possible to identify a threshold dose for cataract induction, or even to conclude whether a true dose threshold is applicable (2, 3). This represents an important current public health issue, especially for medical radiation workers, since many have amended or will need to amend their working practices in response to the new dose limits, such as the EU Basic Safety Standards in EU countries (5).

The LDLensRad project was initiated in 2017 with funding from the EU CONCERT European Joint Project, with the objective to bring together experts from across Europe to answer a number of key outstanding research questions regarding radiation cataractogenesis. These questions included:

1. How does low dose radiation cause cataracts?
2. Is there a dose rate effect for cataract induction?
3. How do age at exposure and genetic background influence cataract development after radiation exposure?

In addition, the project was designed to explore the shape of the dose response more closely over time, the debate regarding the nature of cataract (deterministic, stochastic or both), the identification of biomarkers or bioindicators of global radiosensitivity, and provide education and training for early career scientists.

The overview of the LDLensRad project is described in Fig. 1. The main focus of the experimental campaign was the radiation response of mice of a variety of genetic backgrounds for which radiation sensitivity in terms of

[1] Address for correspondence: Cytogenetics Group Leader, Public Health England Centre for Radiation, Chemical and Environmental Hazards (PHE CRCE), Room C1.32, Chilton, Didcot, Oxon OX11 0RQ, UK; email: liz.ainsbury@phe.gov.uk.
[2] Focus Issue guest editor.

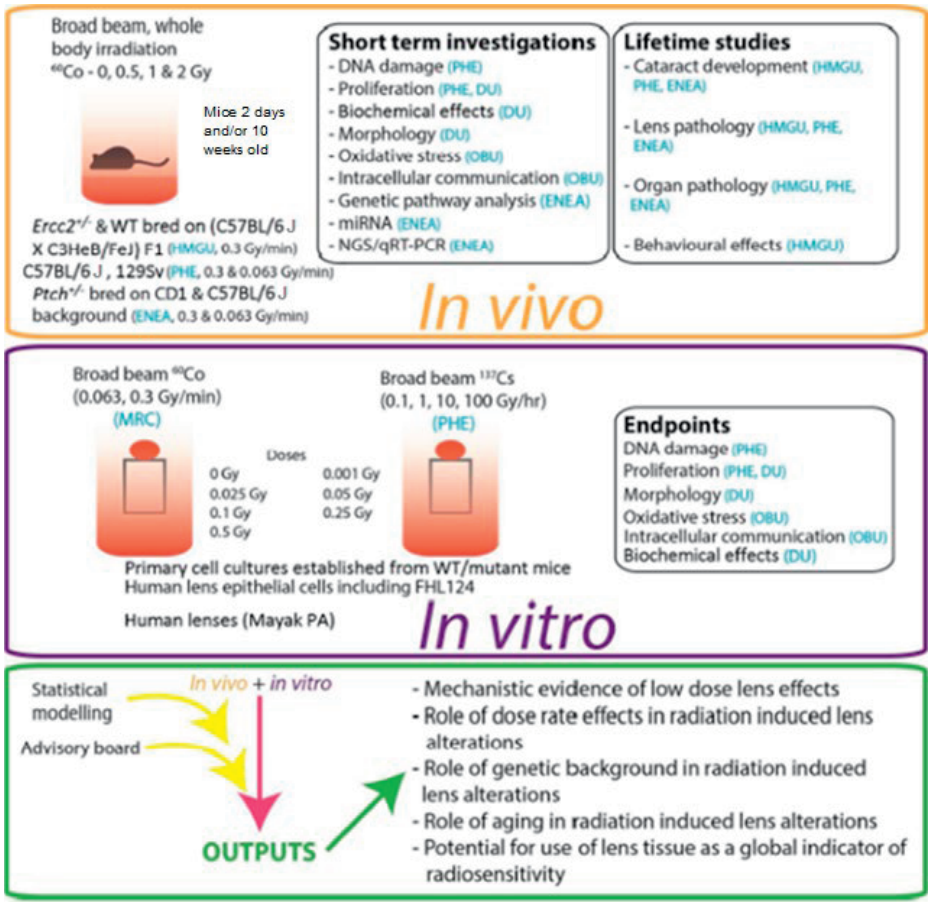

FIG. 1. Overview of the LDLensRad Project. Short term investigations 4 h–12 months postirradiation; lifetime studies 12–18 months postirradiation (full details of doses, dose rates, strains, age at exposure and post exposure time points given in the individual publications).

cataract development was known or suspected. A range of doses and two dose rates were chosen to assess the impact of genetic background. A number of different short- and long-term experimental endpoints hypothesized to be involved in the action of radiation in the lens were chosen for investigation. This work was supported by investigation of additional endpoints using lens epithelial cell (LEC) lines *in vitro* to complement the *in vivo* program. The project was supported by an active Scientific Advisory Board.

This Focus Issue describes some of the key results of the LDLensRad project. However, a number of articles were published in advance of those presented herein, and a very brief summary of those publications is warranted so as to comprehensively describe the scope of the entire project.

- Uwineza et al. (6) sought to re-design the concept of the ''cataractogenic load'' whereby ionizing radiation adds to the burden of cataractogenic changes induced by genetic, lifestyle and environmental factors.
- Barnard et al. (7) found that there was an inverse dose-rate effect for DNA damage repair in the mouse lens after measuring residual 53BP1 foci induced by 0.5–2 Gy at 4 and 24 h postirradiation of mice at 10 weeks of age. This observation was not seen in peripheral lymphocytes obtained from the same mice.
- Pawliczek et al. compared Spectral domain – optical coherence tomography (OCT), histology and Scheimpflug imaging as monitoring tools in unexposed mice of between 5 and 78 weeks of age, and highlighted the value of OCT for anterior lens and eye imaging (8).
- Pawliczek et al. found new evidence of vision impairment in B6C3F1 mice, 8.5 months after 2 Gy exposure at postnatal day 2 (9).
- Quinlan and Hogg highlighted the role of γ-crystallin as an oxidoreductase and in aggregation of mutant crystallins (10).

A lifetime study of radiation-induced catararactogenesis was carried out in male and female *Ptch*1$^{+/-}$, *Ercc*2$^{+/-}$ and C57BL/6J mice (see Fig. 1) irradiated at 10 weeks of age with doses of 0–2 Gy using dose rates of 0.063 and 0.3 Gy min^{-1}. The results, in terms of lens opacification as monitored by Scheimpflug imaging over a period of up to 18 months postirradiation, are reported in McCarron et al. in this issue (11). As expected, background opacification increased with time and dose in most models. Mean lens density increased with higher dose and dose rate in mice

with Ercc2 and Ptch1 mutations, and overall, sex, dose, dose rate and genetic background were all found to be significant contributors to opacification, with strain being the largest single contributor to percentage opacification. Most importantly, significant interactions between these experimental factors were identified for the first time, clearly demonstrating the importance of testing or controlling for sex, genetic background and dose rate in studies looking at the impact of ionizing radiation on cataract formation. As different strains were housed in different locations, further investigation of the husbandry conditions must be carried out to ensure the ''strain'' differences observed for lens opacification are not due to, at least in part, differences in animal housing facilities. It is also important to assess the data in the context of the previous findings of Pawliczek et al., which indicate that Scheimpflug imaging may not be the most ideal method for tracking early radiation-induced opacities, and particularly posterior subcapsular cataracts (PSCs), due to a reported lack of sensitivity for this type of opacity (8).

To further investigate the wider systemic effect of ionizing radiation, De Stefano et al. (12) explored the specific responses in both the lenses and brains of neonatally exposed *Ptch*1$^{+/-}$ mice bred on CD1 and C57BL/6J backgrounds that received whole-body irradiation of 0.5–2 Gy with dose rates of 0.3 or 0.063 Gy min^{-1} at postnatal day 2. The authors identified an inverse relationship between radiosensitivity to induction of lens opacification and medulloblastoma up to 10 months postirradiation, and also between lens opacification up to 6 months and neurogenesis at 6 weeks postirradiation, in the *Ptch*1$^{+/-}$ mutants. Strain differences in cerebellum apoptosis at 4 h postirradiation also highlighted the critical importance of genetic background after exposure to low doses. In this case, dose-rate related effects were not detected, perhaps due to the dominance of the genetic effect. This work further highlights the role of genetic background related individual sensitivity in radiation response.

Pawliczek et al. (13) looked at the *in vivo* characteristics of PSC induced in male and female B6C3F1 mice 70 days after 0.5–2 Gy gamma irradiation was delivered at 10 weeks of age. Spectral domain OCT was used to measure visual acuity prior to histological sectioning for examination of cataract phenotype; this was suggested as an improved method over Scheimpflug imaging. The authors identified three different anterior and posterior lesions, with no significant increase in PSCs for doses <1 Gy. Furthermore, they found that PSCs identified using histology were not necessarily vision impairing. Most importantly, the authors found that early lesions formed in response to ionizing radiation exposure were best characterized by a deterministic model, whereas later manifestation was better described by a stochastic model. This key finding further contributes to understanding of how the impact of ionizing radiation on the lens might be best addressed for radiation protection purposes.

Following a number of publications which have highlighted the potential role of DNA damage and repair within the lens epithelium in cataractogenesis, and the recent findings related to inverse dose rate response (7) in C57BL/6J mice, Barnard et al. further explored DNA damage responses in 10-week-old *Ercc*2$^{+/-}$ and *Ptch*1$^{+/-}$ mice exposed to 0.5–2 Gy gamma radiation, at dose rates of 0.3 and 0.063 Gy min^{-1}. Their observation of a definite, direct dose rate effect for cataractogenesis in both wild-type mice and mice with genetic backgrounds that confer enhanced radiosensitivity further support the hypothesis that the DNA repair response is different in the lens epithelium compared to other tissues (14).

Barnard et al. (15) also looked at Ki67 responses as a marker of proliferation at 30 min, 4, 24 and 48 h, and 3, 7, 10 and 14 days postirradiation in female C57BL/6J mice exposed at 10 weeks of age to the same radiation conditions as the genetic background studies. Radiation increased proliferation of LEC at 2 and 24 h postirradiation, and while dose rate was not identified as a singular significant factor, there was a significant interaction between dose rate and the lens epithelial region, with evidence of increased proliferation with increased dose rate in both the central and peripheral regions (LEC start to differentiate into lens fiber cells in the germinative zone which is found in the peripheral region of the lens epithelium).

Tanno et al. (16) identified different miRNA signatures induced 24 h after 2 Gy gamma irradiation in 10-week-old *Ptch*1$^{+/-}$ mice with different genetic backgrounds using next generation sequencing together with a sophisticated bioinformatics analysis. In particular, the authors identified contra-regulated expression in genes with key roles in regulating Toll-like receptor (TLR) signaling pathways and DNA damage responses involving p53. The interplay between these mechanisms may explain the differences in relative sensitivities of the CD1 and C57BL/6J backgrounds.

Garrett et al. (17) analyzed the effects of 0.5–2 Gy gamma radiation delivered at 0.3 Gy min^{-1} on a range of different animal behaviors in wild-type and *Ercc*2^{S737P} mice. The authors found clear dose-dependent effects, independent of sex or genotype, for a number of endpoints, including spontaneous locomotor and exploratory activity, anxiety-related behavior, body weight and affiliative social behavior. Some genotype, dose and sex-related effects were identified in working memory. For example, locomotor activity (as measured by total distance traveled and average speed of movement) was found to be inversely related to dose with the lower dose enhancing locomotor activity. Most effects were present at 4 months postirradiation and most observations did not persist to 12 or 18 months. It should be noted that at 4 months, lenses in both strains of mice were normal; hence, the observed effects were not correlated with changes in visual acuity. When compared to a previous study at 0.063 Gy min^{-1} which produced

different behavioral outcomes (18), these data provide further evidence that dose rate impacts radiation responses.

In order to further support the *in vivo* experimental findings, Ahmedi et al., investigated mechanisms of cataract induction *in vitro* using immortalized human LEC lines exposed to ^{137}Cs gamma rays at doses of 0, 0.1, 0.25 and 0.5 Gy and at dose rates of 0.063 and 0.3 Gy min^{-1} (19). Cell viability decreased in a dose-dependent manner over 24 h postirradiation as indicated by an increased permeability to DNA staining dye, and reactive oxygen species (ROS) and DNA damage were increased when measured at 1 h postirradiation. Both ROS and DNA damage progressively decreased with increased time after irradiation through 24 hr. Induction of senescence was also observed 15 days postirradiation, but this was not attributed to telomere erosion of the reduction in telomerase activity. The results illustrate the potential for *in vitro* work to support the investigation of low-dose effects in the lenses of living animals. While it is difficult to draw firm conclusions in such cellular models, the data demonstrate slight genetic differences in the reduction in viability in response to radiation exposure. There was also a small but statistically significant indication that the lower dose rate was more effective in reducing cellular viability for some but not all experimental conditions. This mirrors the results of Barnard et al. (4) for the DNA damage response, although others failed to report such a response. Various hypotheses have been suggested to explain these observations, including the failure of LECs to arrest in the G2 phase of the cell cycle phase *in vivo*. These aspects clearly require further investigation. Ahmadi et al. also highlight the need for further work looking at the potential involvement of intracellular signaling (for example through microvesicles and exosomes) and bystander effects as well as the interruption of disassembly of the nuclear envelope in differentiating LECs, as a consequence of radiation-induced senescence (19).

The mechanisms of radiation cataract were reviewed by Ainsbury and colleagues (20) just prior to the commencement of the LDLensRad project. At the time there were several open questions, particularly regarding the influence of strain and dose rate. It is clear that the LDLensRad project has advanced our understanding in this important area of radiation protection. Each of the individual articles in this Focus Issue comment, at least in brief, on the future initiatives for radiobiological research on the lens, highlighting overall the need for continued research in this important area, and in support of appropriate radiation protection for the lens. However, as a direct result of the work of the LDLensRad program, a number of important conclusions can now be drawn. These include, in some of the studies, the detection of measurable damage with very low doses and at very low dose rates. The apparent inverse dose rate effects for some endpoints are also of particular note. These findings are clearly of relevance to occupational exposures and, taken together with the finding of Pawliczek and colleagues that phenotypic changes to the lens can be both deterministic and stochastic, could imply that further work is needed to ensure the system of radiation protection is fit for purpose (21).

As others have noted (22, 23), there is a particularly urgent need for a clearer understanding of how low dose, high-linear energy transfer radiation affects the lens and elicits wider, systemic, effects including those in the retina, brain and other organs.

In conclusion, the authors of this Commentary now strongly recommend the incorporation of cataract studies as part of any larger scale programs focused on multiple radiation health effects.

The authors would like to thank the LDLensRad Consortium members, all of whom have been incredibly active, as well as the large number of supporters – not least Paul Schofield and Michael Gruenberger who helped enormously with STORE, and the CONCERT coordinator and colleagues too.

Finally, we would like to dedicate this Focus Issue to our wonderful colleague Gabriele Babini who passed away unexpectedly in November 2020. He was an integral part of both the planning and the implementation of the project, and we could not have done it without him. He will be sadly missed by many.

IN MEMORIAM

On November 5, 2020, our young colleague Gabriele Babini (top row of Fig. 2, second from left) unexpectedly passed away in Rome, Italy. Gabriele was born in Lugo (Ravenna) on November 20, 1987. In 2009 he obtained a three-year degree in Physics from the University of Trieste, and in 2011 a master's degree in Physical Sciences from the University of Pavia. He obtained his PhD in Physics from the University of Pavia in January 2015 with a thesis entitled ''*Systems radiation biology to unravel radiation-induced dysregulation of cellular pathways*'' and continued his research activities as a research fellow at the same university until December 2018. Since January 2019 he was hired as a research collaborator by the Fondazione Policlinico Universitario Agostino Gemelli in Rome. He is the author of 40 publications in international scientific journals and of many conference presentations. His professional competence, at the crossroads between biology and radiation physics, strongly stimulated Gabriele's interest in the study of bioinformatics applications and systems biology, and in this area he quickly established himself as a point of reference in the Italian and international radiobiological community, working and making himself known and appreciated in various national and European projects, and establishing fruitful collaborations with important research institutions. Gabriele actively participated in the LDLensRad project, making available to the partnership his knowledge of bioinformatics and his

FIG. 2. The LDLensRad Consortium members in Munich, Germany in 2018.

brilliant skills of data analysis and interpretation. He co-authors three of the manuscripts in this special issue.

We want to dedicate this volume to him, expressing deep gratitude for having shared his innovative vision with us and for having passed it on to the younger colleagues. Besides his important scientific contributions, we wish to remember his outstanding human qualities, his cheerfulness and passion for sport and good food that allowed a deep bond of friendship to grow over the years. The memory of the moments spent together and his smile will remain etched in our minds and hearts.

Arrivederci Gabriele!

The LDLensRad Consortium

Received: October 4, 2021; accepted: November 2, 2021; published online: November 17, 2021

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