Eye lens exposure to IR: current understanding, radiobiology and dose limits

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Background

Cataracts are the most frequent cause of blindness worldwide

Multifactorial aetiology: Age related effect; Genetic component (congenital cataracts); Also: Sunlight, alcohol intake, nicotine consumption, diabetes, persistent use of corticosteroids, and ionising radiation…
ICRP defines:

• **Deterministic or tissue effects**

  Those for which there is a defined threshold below which the effect does not occur; severity of effect increases with dose

• **Stochastic effects**

  Those for which there is no threshold, risk (but not severity) increases with dose

http://www.icrp.org/images/P118.JPG
Ionizing radiation is generally (but not exclusively) associated with posterior sub-capsular opacities

Adapted from Beebe, 2008

This and other images without references throughout: Ainsbury et al., 2016
Lens protection

- **Paradigm**: Radiation cataract is a deterministic, late, effect

- **Recent epidemiological (re)analyses**: The lens is more radiosensitive than previously thought

- **ICRP, 2012; EU BSS, 2014**:
  - Threshold for radiation cataract $\sim 0.5$ Gy
  - Occupational lens dose limit $20$ mSv y$^{-1}$ (averaged over 5 years, with no single yearly exposure exceeding $50$ mSv)

- **But**: This is based on epidemiological data…

http://www.icrp.org/images/P118.JPG
How does ionising radiation (at low doses) influence cataract development?
Human Lens

- Diameter ~9-10 mm, thickness ~4.5 mm
- Germinative zone of LEC
- Growth factors
  -> differentiation
  -> lens fibres
- Tight temporal and spatial organisation
- Deregulation -> cataracts
**Target cells:** Germinative Zone on lens epithelium + ...

**Potential mechanisms might include:**
- Oxidative stress
- DNA Damage/Repair/Mis-repair
- Intracellular signalling
- Gene expression
- Cellular proliferation / mobility / migration
- Damage to proteins/ECM/lipids
- Post translational modifications
- Senescence
- Systemic/Non-targeted effects ...

**Modifying factors:** Dose, Dose rate, Age at exposure, Genetic background ...
ROS: Degradation, cross-linking, aggregation of lens proteins, DNA damage

Hamada et al., 2014

-> Aberrant lens epithelial cell division, cell migration, differentiation…
Evidence from a study looking at DNA damage and repair

Markiewicz et al., 2015:
- Low dose, dose-response for DNA damage response in the lens
- Lens is more sensitive than circulating lymphocytes
An inverse dose rate effect for DNA damage

Barnard et al., 2018:

At low doses, lower dose rates induce more DNA damage in the lens but not in blood lymphocytes.
This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 662287.

Signalling: Tumour related factors

Hamada and Fujimichi, 2015
**Stimulation of proliferation**

Fujimichi and Hamada, 2015: “IR not only inactivates clonogenic potential but also stimulates proliferation of surviving unactivated clonogenic HLE cells”

IR -> abnormal activity

Historical data: Irradiation induces excessive proliferation of rabbit lens epithelial cells; suppression of lens epithelial cell divisions inhibits radiation cataractogenesis in frogs and rats

Markiewicz et al. 2014:

At low doses, a non-linear response
Protein modification

Fujii et al., 2001: Role of post translational modifications? May reduce solubility to alter transparency

Bloemendal et al, 2004: Lens crystallins: α-,β- and γ-, form the refractive medium of the lens; proteins e.g. αA- or αB- protect from aggregation

Muranov et al., 2010: Protein changes in irradiated lenses similar to those seen in old age

Wiley et al., 2011: Role of abnormal cellular proliferation, e.g. p53 effect?

New data: Dose, dose rate and exposed region all impact cellular proliferation and morphology…
**Mouse models:** ATM, RAD9, BRAC1 genes control signalling for DNA damage response signalling; Heterozygosity of these genes known to leads to increased risk of cancers

**Worgul et al., 2002:**
- Cataracts earliest in homozygotes for Atm, then heterozygotes, then wildtype.
- Severity and latency proportional to number of damaged cells attempting differentiation.
- Atm homozygotes/heterozygotes – genetic predisposition to cataracts

**Kleiman et al., 2007:** Cataracts develop earlier and in greater numbers in Atm/Rad9 double heterozygotes

**Smilenov et al., 2008:** Atm/Rad9/Brca1 double heterozygotes showed increased resistance to apoptosis and increased radiation sensitivity

**Humans:** e.g. Cataractogenic mutations in human crystallin genes

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E.g. Oxidative stress:
- Proteins: post-translational modifications → aberrant aggregation
- Lipids: lipid peroxidation → elevated hydroperoxides and oxy-derivatives → modifications in lipid-lipid and protein-lipid interactions

Uwineza PhD hypothesis:
Accelerated aging: Ionizing radiation leads to oxidation of major proteins and lipids in the LFCs membrane disrupting eye lens homeostasis

- IR impacts lipid formation
- Genetics influences the impact

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**Ercc2\(^{+/S737P}\)** mice:

- Lens density increased with age
- In 10 week old mice, Scheimpflug imaging revealed no radiation-induced or clinically relevant lens opacifications (note: the posterior lens is not fully visible) up to 18 months
- P2 neonates showed a significantly higher incidence of cataracts. About half of the irradiated mice developed a clear cataract with a lens density over 14%
Ptch1\(^{+/−}\) mice:

- Adult mice: Dose effect was dependent on strain; dose rate effect for Ptch mice only
- P2 irradiated mice showed a clear effect of age at irradiation in accelerating cataractogenesis in Ptch1\(^{+/−}\) and significantly in WT mice on CD1 background, but not on C57BL/6 background
Summary of ‘imaging’ results

• **Significant effects** of dose and dose rate have been detected in some models.

• But, most lens densities at 19 months post exposure were below the LOCS III criterion of 14.1% for a type 2 cataract in human.

• **Age** and **genetics** significantly contribute to cataract risk.

• For most mouse models, the effect of aging and strain outweighs the effect of radiation dose or dose rate.

• Ptch1+/− P2 irradiated mice: The effect of age at irradiation is strongly influenced by genetic background, **clear interaction effects for all strains**.
Hippocampal neurogenesis and behavioural testing

Results: Lens as a global biomarker

Neuronal progenitors 4 months post irradiation

Y-maze entries (8 min)

- Control effect: p < 0.0001
- Genotype effect: p < 0.01

Dalke et al., 2018

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(Very basic) summary of current (incomplete) mechanistic hypothesis

Ionising Radiation

DNA damage

ECM

Oxidation

ROS

Dividing cells

+ + +

Post translational effects

Abnormal differentiation and migration

Radiation cataract

Proteins and lipids

Abnormal accumulation of lens proteins

Genetic effects

Wider / Systemic effects

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Uwineza et al., 2019: Cataractogenic load
What do we know?

- Understanding of lens biology (structure, physiology, process of fibre cell formation)
- Epidemiology: IR is associated with posterior sub-capsular cataracts...
- High dose response
- Involvement of genomic damage of lens epithelial cells
- Morphological changes: Number of potential competing/parallel mechanisms
- Genetic background
- Age dependence (radiation acceleration)
- Cataract detection/assessment
What don’t we know?

- Nuclear/cortical
- Latency period length
- Monte Carlo modeling of dose deposition
- Confounding factors
  - Smoking
  - Alcohol consumption
  - Background IR exposure
  - UV exposure
  - Obesity
  - Diabetes
  - Hypertension
  - Eye injury/inflammation
  - Asthma
  - Steroids
  - COPD
‘Take home’ messages

• Current regulations are based on (mostly) high dose population studies

• The mechanistic link between low dose radiation and cataract is still under investigation (but some excellent studies in progress!)

• ICRP must make pragmatic recommendations to protection radiation workers and the public in spite of a lack of complete information

• EU occupational lens dose limit: 20 mSv y⁻¹ (averaged over 5 years, with no single yearly exposure exceeding 50 mSv)

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Thank you for listening!

Questions / comments?

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