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# EJP-CONCERT

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# D9.47 – In vitro proteomic, biochemical studies

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## Abstract

The lens of the eye is more radiosensitive than previously thought, but the mechanism(s) that lead to low dose radiation cataract have yet to be fully elucidated. This is now a critically important knowledge gap for radiation protection because of the recent substantial reduction in occupational dose limits as a result of epidemiological information and reanalyses. The public health issues concern medical radiation workers, many of whom will likely need to amend their working practices as our understanding of the underlying process and ultimate effects of chronic, low dose, ionising radiation exposure becomes more complete.

The LDLensRad project brings together experts from across Europe to answer a number of key research questions on this topic, including: how does low dose radiation cause cataracts; is there a dose rate effect, and how does genetic background influence cataract development after radiation exposure. CONCERT Deliverable 9.47 of the project, describes the molecular mechanisms through which ionising radiation causes damage to the macromolecules, with the focus on lipids, and how this can lead to cataractogenesis. The data are currently being collated and will be analysed for submission for publication in due course.

# Content

# Progress summary

## 1 Introduction

The eye lens is one of the most radiosensitive tissues in the human body and for that reason is often studied regarding non-cancer effects of ionising radiation (IR) on cells. The eye lens is comprised almost entirely of differentiated fibre cells (LFCs) and because of the lens structure and biology in the mature fibre cells, there is mechanism for cell removal and so these cells contain some of the oldest proteins and lipids in our bodies. It is, however, the single layer of epithelial cells (LECs) covering the anterior half of the lens, and in particular those at the equator of the lens, that have been identified as the target for acute IR damage. Low dose IR effects involve a long latency before the manifestation of cataracts. LFCs and their long-lived proteins and lipids are also implicated in IR-induced cataracts.

Epithelial cells located at the lens equator begin to differentiate and are positioned via the meridional rows to form LFCs, which are then retained throughout life (1). LFCs continue their process of differentiation and this includes the degradation of their intracellular organelles and DNA, and with this the capacity to synthesize new proteins is lost. The youngest LFCs are found in the lens cortex whilst the oldest LFCs, including those formed prenatally, are situated in the centre of the lens, which is known as the lens nucleus. In order to provide the required refractive properties, the lens cytoplasm contains proteins called crystallins at very high concentrations, which depending on the species, can be up to 0.6 mg/ml. Besides these specialised proteins, the lipid composition of LFC membranes are also adapted to not only reduce oxygen tension, but also to support the function of integral membrane proteins such as the water channel AQP0 that is essential for lens accommodation (2). With age, the crystallins aggregate and accumulate on the lens membrane and AQP0 is truncated (figure 1, from Uwineza *et al.* 2019 (3)), causing presbyopia, but as crystallin post-translational modifications accumulate, then cataract formation is initiated and oxidative damage increases.

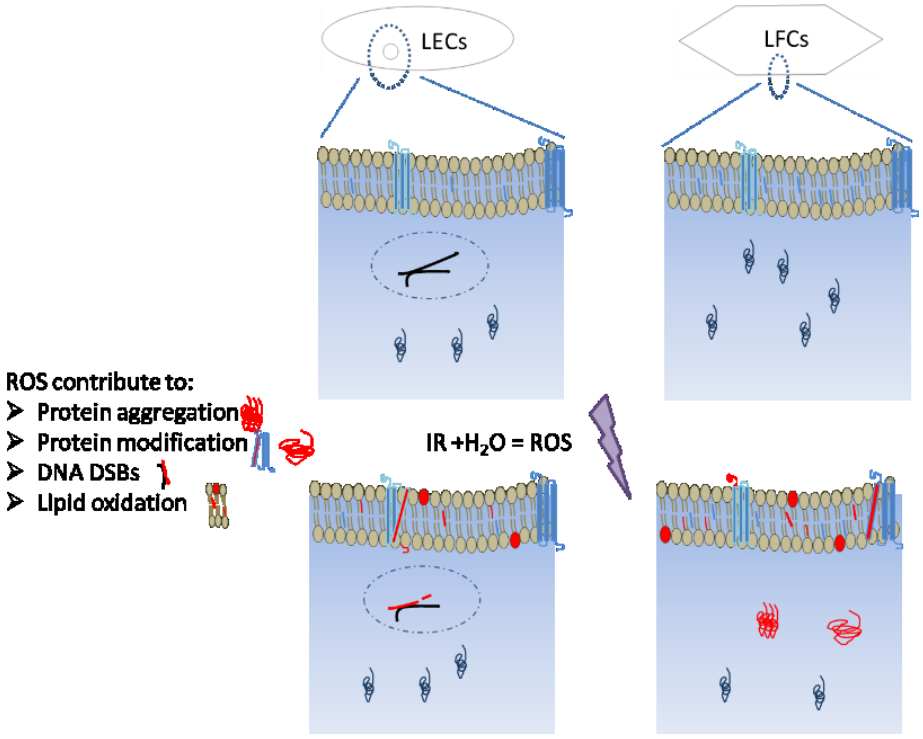


*Figure 1: IR-induced oxidative stress causes damages to all macromolecules in the cells.*

The plasma membranes of the LFCs are quite unique containing the highest proportion of cholesterol in comparison to other cell types in the human body. Cholesterol levels increase with age and cholesterol lipid rafts are a key feature of LFC membranes (4). These lipid raft regions are also enriched in AQP0 and membrane bound crystallins. Changes in the lipid composition of LFC membranes have been shown to affect the functionality of these proteins. So the lipid composition is also an important parameter for lens homeostasis.

The importance of these highly cholesterol-concentrated clusters in the eye lens is being investigated. Interestingly, increased levels of lipid peroxidation products have been observed in lenses of cataract patients compared to age-matched controls (5). Lipid peroxidation is also a consequence of IR exposure and epidemiological data reveal a correlation between IR exposure and the development of eye lens cataracts (3). We therefore hypothesize that IR leads to the conversion of cholesterol into oxysterols, which we suggest contributes to cataractogenesis by disrupting plasma membrane cholesterol homeostasis.

# 2 Methods

To study IR-induced lipid peroxidation *in vitro* the isolated bovine eye lenses (from an abattoir so with no further information) were collected and decapsulated. The nucleus and cortex were separated by aqua-dissection and the lipid membranes of the LFCs produced as described (Tapodi *et al.* 2019). These membrane fractions were subsequently exposed to X-rays in a dose dependent manner up to 50 Gy. Lipid purification was performed using the Bligh-Dyer method. A similar approach was used to study cholesterol oxidation *in vivo* (6). Mice were exposed to X-ray doses up to 4 Gy. Eye lenses were collected using a dissection microscope. Analogous to the bovine *in vivo* samples, lipid membrane extraction and lipid purification was executed with these mouse samples.

# 3 Results

We observe that most of the increased cholesterol oxidation products in age-related cataracts were oxysterols such as 7-keto cholesterol, which showed the highest increase after IR exposure. The results are currently being collated and prepared for submission for publication.

# 4 Preliminary Discussion

IR increases oxidative stress in cells through the production of free radicals via the radiolysis of water. These free radicals can react with the proteins in the cytoplasm. Even though slightly less accessible for water molecules, reactive oxygen species migrate into the plasma membrane and also damage the proteins and lipids in this hydrophobic environment.

The presence of high levels of cholesterol in these membranes makes it an easy target for X-ray generated free radicals. They induce free radical chain oxidation leading to the formation of cholesterol oxidation products. Oxysterols can be formed by either enzymatic reactions, e.g. 25-hydroxy cholesterol or as a result of auto-oxidation (e.g. 7-keto cholesterol) (5). Given that metabolic activity in LFCs is very low in comparison with LECs, we expect most of the oxysterol formed will be through the auto-oxidation pathway. Disruption of cholesterol homeostasis not only interferes with the integrity of the plasma membrane, but also with functional properties of the proteins. This will be further investigated by analysing the results of this work together with the wider LDLensRad endpoints as the project progresses.

The information presented in this deliverable represents methodological development towards investigation of lipid peroxidation. High doses of radiation have been deliberately applied in order to first develop methods to allow measurement to take place – these will later be reduced as the methods are refined to investigate lower doses relevant to exposed human populations.

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