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PII: S1383-5742(18)30081-4
DOI: https://doi.org/10.1016/j.mrrev.2019.02.004
Reference: MUTREV 8264

To appear in: *Mutation Research*

Received date: 18 August 2018
Revised date: 12 February 2019
Accepted date: 14 February 2019

Please cite this article as: Uwineza A, Kalligeraki AA, Hamada N, Jarrin M, Quinlan RA, Cataractogenic load – a concept to study the contribution of ionizing radiation to accelerated aging in the eye lens, *Mutation Research-Reviews in Mutation Research* (2019), https://doi.org/10.1016/j.mrrev.2019.02.004

CATARACTOGENIC LOAD – A CONCEPT TO STUDY THE CONTRIBUTION OF IONIZING RADIATION TO ACCELERATED AGING IN THE EYE LENS

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Competing Interests: The authors have no competing interests to declare.

ABSTRACT

Ionizing radiation (IR) damages DNA and other macromolecules, including proteins and lipids. Most cell types can repair DNA damage and cycle continuously their macromolecules as a mechanism to remove defective proteins and lipids. In those cells that lack nuclei and other organelles, such as lens fiber cells and mammalian erythrocytes, IR-induced damage to macromolecules is retained because they cannot be easily replenished. Whilst the life span for an erythrocyte is several months, the life span of a human lens is decades. There is very limited turnover in lens macromolecules, therefore the aging process greatly impacts lens structure and function over its lifetime. The lens is a tissue where biomolecular longevity, lifelong retention of its components and continued growth are integral to its homeostasis. These characteristics make the lens an excellent model to study the contribution of retained macromolecular damage over time. Epidemiological data have revealed a significant association between exposure to IR, the loss of lens optical function and the formation of cataracts (cataractogenesis) later in life. Lifestyle, genetic and environmental factors all contribute to cataractogenesis due to their effect on the aging process. Cataract is an iconic age-related disease in humans. IR is a recognised cause of

ABBREVIATIONS: IR: Ionizing radiation, PSC: posterior subcapsular cataract, ARC: age-related cataract, A-bomb: atomic bomb, DSBs: double strand breaks, mtDNA: mitochondrial DNA, ATM: ataxia telangiectasia mutated, ATR: ataxia telangiectasia and Rad3-related, BER: base excision repair, NER: nucleotide excision repair, LECs: lens epithelial cells, AGE products: advanced glycation end products, sHSP: small heat shock protein, LMDP: large molecule diffusion pathway, ROS: reactive oxygen species, FGF: fibroblast growth factors, NASCA: NASA Study of Cataract in Astronauts, ICRP: International Commission on Radiological Protection, SSBs: single strand breaks, RIBE: radiation-induced bystander effect, LFCs: lens fiber cells, ND: not determined

cataract and the occupational lens dose limit is reduced from 150 to 20 mGy / year averaged over 5 years (ICRP Publication 118). Understanding the effects of low dose IR on the lens and its role in cataractogenesis is therefore very important. So we redefine "cataractogenic load" as a term to account for the combined lifestyle, genetic and environmental processes that increase biomolecular damage to lens macromolecules. These processes weaken metabolic defenses, increase post-translational protein modifications, and alter the lipid structure and content of the lens. IR exposure is a significant insult to the lens because of free radical generation and the ensuing oxidative stress. We support the concept that damage caused by IR compounds the aging process by increasing the cataractogenic load, hereby accelerating lens aging and its loss of function.

Keywords: Eye lens, cataract, ionizing radiation, posterior subcapsular cataract, aging, double strand breaks, reactive oxygen species, lipid peroxidation

1 INTRODUCTION

Ionizing radiation (IR) exposure induces an iconic eye pathology, namely lens cataract [1, 2]. The lens patho-phenotype typical of a previous IR insult is reported to be a particular type of cataract, namely posterior subcapsular cataract (PSC; [3-5]), but cortical and nuclear cataracts are also reported [4]. PSC is also not unique to IR exposure as it is associated with aging, although nuclear cataract is the most prevalent phenotype of age-related cataract (ARC) [6-9]. A mechanistic explanation has been proposed for nuclear cataract based on an age-related change in lens physiology and a consequential dramatic increase in the oxidation of nuclear lens proteins [10].

Here we reconsider the concept of "cataractogenic load" to explain the process of IR induced cataractogenesis. We redefine the concept as originally proposed [11] to now include damage not only to the epithelial cell genome, but also to all biomolecules in the lens, independent of whether this be caused by IR or age-accumulated modifications to the proteins, lipids and DNA in lens cells. This reflects recent data on protein [12-14] and lipid stability in the lens [15-17], the proposed large molecule diffusion pathway [18] and the accumulation of metabolites, derived from tryptophan [19, 20], glutathione [21], sugars and carbonyl compounds [22, 23] that arise in an age-dependent manner. Both proteins and lipids in the lens accumulate modifications as the individual ages [10, 14, 15] and IR is another potential cause of these protein and lipid modifications. This is in addition to any DNA damage sustained by nucleated lens cells [24]. Here we redefine cataractogenic load

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as a term to describe the accumulated damage to DNA, proteins and lipids that may collectively contribute to the eventual development of a lens cataract.

We discuss how exposure to low dose IR contributes to the development of lens cataract later in life and why this apparently favours PSC rather than nuclear cataract. Previous studies have not shown significant association between IR exposure and nuclear cataract in later life [2, 5], but a study of the atomic bomb (A-bomb) survivors evidenced that younger people (<30 years; mean age at the time of exposure 10.5 years [4]) were more susceptible to PSC [4, 25] and cortical cataract [4]. Most of the IR study groups, namely the Chernobyl cleanup workers [5], those treated for haemangioma in their early childhood [26] and the Mayak Production Association workers [27] were exposed to IR as juveniles or adults in the third and early fourth decade of life. As the latency for cataract development after a low dose IR insult can involve one to three decades [5,27], it is time to re-examine the connection between IR exposure, aging and the PSC phenotype in order to advance our understanding of the mechanisms involved in low dose IR-induced cataract. In the field of radiation protection, low dose has widely been defined as <0.1 Gy [28], but for the purpose of this review, we define low dose as <0.5 Gy according to the latest threshold for cataracts recently recommended by the International Commission on Radiological Protection (ICRP) [29].

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2 PHYSIOLOGICAL AGING

2.1 Aging hypotheses and mechanisms

Normal cell and tissue homeostasis is continually challenged throughout life. To maintain homeostasis, there are many molecular pathways to repair, eliminate and/or replace damaged molecules, cells and tissues [30]. With age, the ability to adequately respond to the environmental and internal stresses is dramatically reduced [31]. Consequently, the damage to nucleotides, proteins and lipids is not properly repaired, and damage accumulates with increasing age [31, 32]. The reduced ability to respond to such stresses, the presence of time-dependent cumulative damage, the loss of homeostasis and resulting increased susceptibility to disease are the "classical" definition of aging [31-33]. The generation of free radicals capable of damaging proteins, lipids and nucleotides [31, 34, 35] was proposed to drive aging and therefore ultimately determine lifespan [36]. This is the oxidative stress hypothesis [37] that later included other free radicals [38] as part of the more general concept that the accumulated damage shortens lifespan [39, 40]. The original free radical concept was later broadened to include the impact of free carbonyls [41] and more recently the possibility that metabolic activity *per se* is a key factor as evidenced by the expansion with age of what has been termed "the deleteriome" [42]. Thus, there is potential for cellular metabolites themselves to drive macromolecular damage in an age-dependent manner [39]. Indeed the inter-relationship between aging and age-related diseases (ARDs) gives credence to the view that aging itself is a disease as the mechanisms that underpin aging also drive ARDs that shorten lifespan [43]. A logical conclusion from such concepts is that ARDs arise via accelerated aging. For the individual, it is both their genetic and environmental background as well as lifestyle that determine the rate of stress adaptation, proteostasis, stem cell exhaustion, metabolic deregulation, macromolecular damage, epigenetic modifications and inflammation which will ultimately determine how quickly a disease threshold for a particular tissue system is reached during an individual's lifespan [44].

Free radicals can also be produced by external factors (e.g. X-rays); however as they are formed they continuously challenge the integrity and stability of DNA [45], proteins and lipids [46]. This damage is often repaired or countered by cellular defenses. In the case of DNA, there is a complex network of repair mechanisms [47]. The genetic lesions arising include point mutations, translocations, chromosomal aneuploidies, telomere shortening [48, 49]. DNA is the only macromolecule that relies on repair of existing molecules through the whole life of the cells [50]. Unless DNA damage is repaired, the DNA mutations can influence the expression of essential genes and transcriptional pathways, resulting in dysfunctional physiology and accelerated aging [33, 51]. Recognition of DNA double strand breaks (DSBs) involves Ku70/Ku80 or Mre11/Nbs1/Rad50 complexes [50, 52, 53]. The initiation of the downstream repair pathways requires activation of ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) kinases [54] followed by phosphorylation of histone H2AX [55] and other downstream targets including checkpoint kinases 1 and 2 and ligase IV/XRCC4 complex [56]. Damaged bases and nucleotides are repaired by base excision repair (BER) and nucleotide excision repair (NER) pathways [57, 58]. The genomic stability systems also include specific mechanisms for maintaining the appropriate length and functionality of telomeres and for ensuring the integrity of mitochondrial DNA (mtDNA) [59, 60].

In proteins, oxidative stress causes oxidation of amino acid side chains, fragmentation of polypeptide chains, generation of cross-links and conformational changes. These oxidative modifications are usually irreversible and lead to serious disruption of protein function [61], attracting the attention of protein chaperones [62, 63]. The solution appears to be to continually turnover proteins [63], removing the damaged proteins via the autophagy and/or proteasome pathways [62, 64, 65]. This works for and is sustainable in transcriptionally active cells, but is not the case for denucleated or organelle-free cells such as mature erythrocytes and lens fiber cells (LFCs) where protein damage still occurs. Whilst erythrocytes are replaced regularly, the lens is a closed system that retains all its fiber cells throughout the entire lifetime of the individual.

Oxidative stress also damages lipids [66, 67]. Lipids are important structural components of cell membranes but they can also serve as an energy reserve [68]. Lipid homeostasis and metabolism are key to understanding lifespan and disease [69-71] and are directly influenced by lifestyle [71]. A wide range of diseases involving lipids and their derivatives have been described [72-76] and are also linked to cataract [77-81]. Such observations link lipids, lifespan and the lens.

Generic anti-oxidant and free radical defence systems are available in cells. Anti-oxidants such as glutathione, vitamins C and E as well as enzymes to produce these antioxidants and remove free radicals include superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase [82]. During aging the balance between oxidants and the mechanisms that protect against oxidation changes and the numbers of damaged proteins and lipids increase [31, 32].

2.2 The contribution of genetics to aging

The influence of genetic factors in human longevity is limited to just 20–30% [83] of total aging insults [84-86]. Significant differences in aging and lifespan have been described between monozygotic human twins [87-89], and diet/ lifestyle is but one potential modifier [70, 71, 90]. This still leaves 70–80% of the aging process to non-genetic, stochastic environmental factors. It is well established that these environmental stressors induce epigenetic changes in genetically identical individuals [91-94]. These epigenetic changes due to moderate stress induce expression of genes responsible for stress-resistance delaying the aging process. However, severe stress causes an increase in accumulation of physiological damage and abnormalities, accelerating the process of aging [95, 96].

This means that chronological age and biological aging can differ. The biological aging process involves interaction between genes, proteins and their products, environmental stressors and diet throughout the life of the individual. The combination of internal and external stressors, diet and life style are considered to be the main factors contributing to accelerated biological aging, which results in increased susceptibility to pathologies associated with aging, and decreased longevity [43, 97, 98].

3 LENS BIOLOGY AND ITS AGING FEATURES

The vertebrate ocular lens is an avascular, transparent organ which refracts the incoming light onto the retina in partnership with the cornea. The lens itself comprises a lens capsule, a single layer of lens epithelial cells (LECs) covering the anterior half of the lens (Figure 1)

and lastly the LFCs, which differentiate from LECs are produced in the germinative zone at the lens equator (reviewed in [99]). The development and maintenance of optical transparency, refraction and elasticity is crucial for the physiological function of the lens. All change as a result of aging, with presbyopia (gradual deterioration of the ability to focus on close objects) being the universal phenotype experienced by everyone who reaches their fifth decade of life [100]. Presbyopia represents a major functional compromise of age-dependent lens function (Figure 2). Importantly, the lens is a closed system [101] where proteins and lipids are retained from lens formation during development in utero to the point of death.

To ensure lasting lens transparency, maturing LFCs undergo complete loss of organelles such as the cell nucleus, mitochondria and endoplasmic reticulum [102] and accumulate a remarkably high concentration (up to 600 mg/ml) of crystallin proteins which not only form the lens refractive index gradient, but also provide the chaperone complement of the lens (reviewed in [102]). Due to the absence of organelles in mature lens fibers, the need for nutrients, water, and ions is satisfied via a unique lens circulation system [103]. During fiber cell elongation, cells undergo extensive cytoskeletal remodelling (reviewed in [99]) and establish cell to cell communications through the expression of the water channel aquaporin 0 (AQP0) and the connexins Cx50, Cx46 and Cx43 that form gap junctions [100, 104].

To aid lifelong transparency and optical function, the lens also utilises a regulation system to protect itself against oxidative stress [105-108] on both a proteostatic level and via age-related changes in the membrane lipid composition to reduce oxygen levels in the lens nucleus [109, 110]. The main anti-oxidant enzymes are superoxide dismutase, catalase, glutathione redox cycle enzymes and peroxiredoxins. Superoxide dismutase converts superoxide radicals into oxygen and hydrogen peroxide. Catalase, glutathione redox cycle enzymes and peroxiredoxins collaborate to convert hydrogen peroxide into water molecules [111]. Reduced glutathione is newly synthesized in the lens epithelium and outer cortical fiber cells [112], and functions as the prime anti-oxidant agent by diffusing to the lens nucleus through gap junctions [113].

Tissues like the lens [10] and the erythrocyte [114] are valuable models of aging as they reveal key biological principles in a cellular background partially independent of genetic regulation. As most of the cells comprising the eye lens lack nuclei and biosynthetic organelles (reviewed in [99]), it is an excellent model to study the impact of retained macromolecular damage on aging. Long lived proteins are a feature of many cells and tissues, their turnover being related to both their structure-function role and to their cell environment [115]. Indeed, the lens relies on particularly long-lived, abundant proteins (e.g., crystallins) for its optical clarity and function [12, 13, 63]. Therefore, preservation of homeostasis is performed in a protein environment which accumulates post-translational modifications as a function of age [10].

3.1 The importance of aging in cataractogenesis

As the lens is a closed system [101] where protein turnover is very limited, it is a tissue where most of its protein [12, 13] and lipids [15-17] are as old as the lens itself. As a point in case, proteins in the lenses of the Greenland shark are retained for at least 272 years [116]. Aging of the lens macromolecules is therefore within a unique cellular environment and whilst lens function is maintained, these macromolecules are continually being

compromised. For example, after 5 decades, lens stiffness increases so that near vision is compromised [117, 118]. Subsequently ARC develops with three possible phenotypes – nuclear, posterior subcapsular and cortical cataract [6, 7, 119, 120]. Of these, nuclear cataract is the most prevalent (up to 50%), although PSC still represents a significant proportion among certain population groups (~11%; [7, 121]). Cortical cataract had a higher incidence in younger people (<50 years old; [6, 119]), suggesting that the cortex of the lens could be more vulnerable in an aging context. As the individual ages, the lens changes too. The latency for ARC by definition is decades and coincidentally the appearance of PSC/cortical cataract after exposure to low dose IR as reported for A-bomb survivors can also be decades, although PSC rather than nuclear cataract is more frequent [4]. These observations require further investigation to connect cataract phenotype, age of incidence, and particular environmental stressors.

3.2 Contribution of aging to cataract formation

Genetics, lifestyle and environmental factors all influence aging of the individual [122] and it has proven difficult to capture this within a single theory. Previous theories of aging have included the programmed theory [123], the evolutionary theory of aging with its key concepts of mutation accumulation and antagonistic pleiotropy as proposed by Medaware and Williams respectively, the free radical theory of aging [36], the disposable soma theory [124] and lastly the hyperfunction theory of aging [125]. The recent proposal of a "deleteriome" accounts for the varied contribution of genetics and environment in the aging process and incorporates aspects from all previous theories [122]. We propose "cataractogenic load" as the equivalent for the lens and the process of cataractogenesis (Figure 2), given that the lens is a tissue where biomolecular longevity (Stewart et al 2013; Hughes et al 2016), lifelong retention of lens components and continued growth are integral to lenticular homeostasis [118]. The genetic and environmental influences upon cataractogenesis are well documented [126]. The phenotypic heterogeneity between families or even between family members with the same autosomal dominant mutation evidences multiple mechanistic influences upon the cataract phenotype [126]. As the lens ages so its metabolism changes too, not only in its response to growth factors [127], but also as the result of the buildup of metabolites derived from tryptophan breakdown to form kynurenine derivatives [19, 128], glutathione breakdown [21] as well as the increase in advanced glycation end (AGE) products [22, 23]. These metabolites are all capable of post-translational modifiying lens proteins and this is in addition to the racemization/isomerization events that occur in long lived proteins including lens proteins [14].

The term "cataractogenic load" acknowledges this complexity of the lens aging process and encapsulates the importance of accumulated damage to nucleotides, proteins and lipids [10, 24, 129] and the metabolic changes that contribute to the lens deleteriome as contributory mechanisms in cataractogenesis. This is especially relevant to the development of ARC [6, 7, 119, 120] and low dose IR-induced cataract ([29]; Figures 2 and 3), both requiring long latency periods.

3.2.1 Age-related changes in the lens epithelium, its genome and exome

The logarithmic increase in lens size continues until birth and shortly afterwards lens growth becomes linear in humans [101]. The underlying mechanism for this sudden decrease in cell proliferation is not fully understood. Fibroblast growth factors (FGF) levels, cell proliferation and cell density in the central and germinative zones decrease in

an age-dependent manner [127, 130]. Similar changes in epithelial cell density are seen in other mammals [131, 132] and primates [133]. Some have proposed that increased oxygen levels also contribute with increasing age [134]. The integrity of the meridional rows deteriorates with age, becoming more disorganized in older humans [131]. Coincidentally, similar changes occur after IR exposure (15 Gy) of lenses in young animals [135].

In additon to these gross changes in epithelial cell density, both DNA damage [136] and an age-dependent decrease in the expression levels of DNA repair genes occur in LECs [137, 138]. The resulting accumulation of DNA damage is thought to be associated with the development of ARC [24]. Indeed, changes induced by ultraviolet B (UVB) exposure can repress the DNA repair gene *ERCC6* via hypermethylation [139]. Telomeres are also shortened in cataractous lens samples [140], suggesting a link between ARC and an increase in oxidative stress [141] and perhaps also a link with senescence [142]. Lifelong exposure to UV light is associated with accumulated oxidative stress and is thought to be a factor in ARC [19, 20, 143]. Oxidative stress triggered DNA damage requires repair by BER and NER pathways [144]. In addition to known risk factors in the development of ARC [145-149], there are also positive lifestyle influences that delay its development, e.g. exercise [147] and diet [150], but the phenotype of both ARC and congenital cataract will be subject to the influence of genetic, metabolic and environmental factors [126].

3.2.2 Aging of lens proteins

The lens contains the highest concentration of small heat shock protein (sHSP) chaperones (α-crystallins) among all mammalian tissues [151, 152]. Their role is to prevent protein aggregation, primarily of other crystallins [153]. The lens contains some of the oldest proteins in our bodies [12]. A large molecule diffusion pathway (LMDP) operates in the lens [18]. An exchange of proteins in the soluble fraction between the oldest (central; nuclear) and youngest (peripheral; cortical) cells and vice versa in the lens is suggested from these data [13] possibly as an LMDP [18] component and despite the development of lens barrier in later life [112]. Transfer of some of the proteostatic load accumulating in the oldest lens cells to those with better proteostatic [154] and biosynthetic capacity [155, 156] would be advantageous as suggested from the latest analysis of protein age in the lens [13]. The proteasome [154, 157, 158], calpain [159] and autophagy [160] systems all contribute to proteostasis in the lens.

As lens proteins are long-lived, some amino acids spontaneously decompose (e.g. racemisation; deamidation [14]) due to their intrinsic instability. Although they are also modified by cellular metabolites, this is an order of magnitude less than racemisation and deamidation [10]. To put this in perspective, the proteins in old human lenses have a very low AGE content [10]. Deamidation on the other hand can correlate with increased insolubility [161], reduced chaperone surveillance [162] and with ARC [162].

Damage to the most prevalent proteins in the eye lens, crystallins, is mainly caused by oxidative stress [163]. Reactive oxygen species (ROS) react with crystallins and other lens proteins (Figure 3), contributing to the age-dependent increase in post-translationally modified proteins [163, 164]. Accumulation of these modifications results in protein aggregation (Figure 3), precipitation and eventually the loss of lens transparency and function [165]. At the start of the fifth decade of life, there are significant changes in the lens proteome, the membrane association of previously soluble lens proteins, and an increase in lens stiffness, all of which coincide with the development of presbyopia ([117]; Figure 2).

At the same time, investigation into the correlation between aging and the glutathione gradient in the eye lens showed that glutathione diffusion from the cortex into the nucleus is impaired in older human lenses compared to young lenses [112]. A barrier develops effectively isolating the nucleus of the lens and compromising the reductive defence system of this region [117]. This is one of the paradoxes regarding glutathione homeostasis in the lens [107] as it is unexplained why small anti-oxidants like glutathione are unable to diffuse freely into the lens nucleus, whilst proteins appear capable of slow exchange [13, 18].

Overall the levels of superoxide dismutase, glutathione redox cycle enzymes and peroxiredoxins have been shown to decrease with age, which allows ROS to accumulate in the lens [111, 166]. This is despite the fact that the lens has a well-developed redox regulation system to protect itself against oxidative stress [108]. Also, age-related changes in the membrane lipid composition reduce oxygen levels in the lens nucleus [109, 110].

3.2.3 Age-related changes in lens lipids

Another vital component for the maintenance of lens transparency and preventing oxidative stress induced damage in the lens nucleus is the lens lipidome. The majority of the phospholipids in human lens membranes are phosphatidylethanolamine, sphingomyelin, and dihydrosphingomyelin, of which dihydrosphingomyelin is the most abundant [167]. The human lens has the highest cholesterol concentration among all tissues [168]. With age, the levels of sphingomyelin and dihydrosphingomyelin increase while the levels of glycerolipids such as phosphatidylethanolamine decrease [167]. Ceramides increase dramatically in people older than 30 years of age, whilst glycerophospholipids (with the exception of lysophosphatidylethanolamines) decline rapidly and are almost depleted in people aged 40 years [16]. Cholesterol levels also increase with age [169]. Glycerolipids are less saturated than dihydrosphingomyelin and therefore more sensitive to oxidative stress [170]. Besides unsaturated glycerolipids, cholesterol is oxidized and its levels correlate with ARC [129], again indicating how IR-induced ROS can also potentially modify the lens lipidome (Figure 3). Oxidized lipids trigger changes in the membrane lipid interactions, which lead to disruptions in membrane structure [171]. These disruptions are recognized by phospholipase A2 and removed [172]. It has been hypothesized that the degraded oxidized lipids are replaced by dihydrosphingomyelin and cholesterol, which results in more rigid membranes and increases light scattering [167, 173, 174]. Sphingomyelins are long lived and appear not to turnover in the lens nucleus [17]. Sphingomyelins are thought to increase resistance to oxidation in the lens nucleus of long-lived mammals due to their stable, saturated side chains [109]. Equally, cholesterol domains are also important in reducing oxygen diffusion in the lens [175].

Changes in membrane composition affect interactions with proteins and vice versa [176]. AQP0 is the most abundant protein in lens membranes, and its structure and function are lipid-dependent [177]. The increase in cholesterol and sphingomyelin has been shown to cause a decrease in the permeability of AQP0 water channels [178]. The binding of α-crystallin to the membrane also decreases with increasing cholesterol levels [179]. How all these age-related changes in lipid composition affect AQP0 and α-crystallin binding remains to be elucidated. Nevertheless, there is a correlation between the accumulation of certain oxidized cholesterols and ARC [129] evidencing that changes in the lipidome of lens membranes accompanies cataractogenesis. Oxysterols affect the stability of lenticular protein chaperones [180] and can reverse ARC in some animal models [181], but they also activate small GTPases with consequential changes in the lenticular cytoskeleton [182] and

gap junction-mediated intercellular communication [183]. Lipids are therefore active modulators of lens cell physiology.

3.3 Cataract is primarily an age-related disease

Cataract is one of the main causes of blindness around the world. The age of onset is usually used to classify cataract [126, 184]. Although congenital, juvenile and pre-senile (before the age of 45) cataracts are observed [185, 186], ARCs or senile cataracts are the most prevalent type of cataract with population studies indicating an ARC incidence up to 85% of the cataract affected cohort [187]. The Beaver Dam Eye Study, involving 4926 individuals between the age of 43 and 84 years, demonstrates a direct correlation between cataract incidence and increasing age [7, 188]. Congenital cataracts tend to be caused by mutations in genes coding for proteins vital for lens structure and membrane transport including crystallins [126]. Although genetics have been implicated in ARC pathogenesis [189, 190], lifestyle choices such as smoking [191], alcohol consumption [192] and the individuals environment specific circumstance (e.g. exposure to sun light [19, 193, 194]) are also key factors affecting the age of onset. A study on the involvement of genetics in congenital cataracts and ARC suggests that a limited number of genes have a direct association with increased ARC risk and that these gene mutations result in pleiotropic ARC subtypes [190, 195]. Major genes identified are GALK1, EPHA2, and CRYAA, though others such as GSTM1, and the DNA repair genes WRN and XPD show inconsistent association and penetrance [190, 195-199].

4 THE LINK BETWEEN IR EXPOSURE AND AGING

4.1 Exposure to high IR doses leads to presenile cataract

IR is an important environmental factor in cataract development and the etiology of many other diseases. The effect of high dose IR on human physiology has been widely studied [200-203]. The biological response to high IR dose includes oxidative stress, senescence, and activation of genes linked to aging [204, 205], indicating that high IR doses are a premature age stressor. Research on the physiological, cellular and molecular responses of the eye lens to IR has been ongoing since the discovery of X-rays in 1895 [206] and the associated cataract phenotype [207]. Early studies of IR-induced cataracts that developed in Hiroshima and Nagasaki A-bomb survivors after 6–30 months [3, 208, 209] noted the formation of a central opacity that could adopt a doughnut-like appearance with a diameter of 3–4 mm [209]. Granular opacities and vacuoles sometimes also appeared in the anterior subcapsular region. The initial opacities also had a yellowish (bronzed) hue [209]. The age range of these survivors, who were within a 1000 m of the hypocenter, was between 13 and 50 years. As all the other individuals within this zone died from radiation sickness, shielding by inanimate objects secured survivors. This indicates high dose IR-induced cataracts have a short latency period and lead to presenile cataract [209]. A-bomb survivor studies also considered whether the age of the individual affected the appearance of axial and PSC opacities. For older individuals (>70 years), there was a negative correlation with age for axial opacities, suggesting that the risk was minimal for this cataract type, however a positive correlation was observed for PSC [25, 210] and cortical cataract [4]. Limited histological analysis of IR-induced cataract were gathered from A-bomb survivors, cyclotron workers and those undergoing radiotherapy for a range of ocular tumors and conditions including retinoblastoma [208]. These revealed a significant disorganization and multicellularity of the lens epithelium particularly in the equatorial region, the loss of

meridional row organization and a breakdown in the lens epithelial layer compartmentalization. Similar histopathological changes are observed in other acute insults such as steroid treatment [211, 212] noting that PSC can be caused by a variety of methods and medical conditions [121]. These observations and the transient nature of some PSCs [213-215] are evidence to suggest that the cortex is more sensitive to acute changes in environment and physiology than the lens nucleus.

4.2 Acute versus protracted or chronic IR exposure and lens cataract

Whilst A-bomb survivors received acute doses, people are usually subjected to low dose protracted or chronic exposures. For instance, aircraft crew, cardiologists and radiologists all receive occupational exposure to protracted IR doses. After the Chernobyl nuclear power plant accident, the protractedly exposed liquidators were followed up and the incidence of PSC and cortical cataract reached 25% 14 years post-exposure to doses up to 1 Gy [5]. A comparison of cataracts in astronauts and military aviators showed higher cataract incidence in astronauts [216]. The cataracts of military aviators were mainly PSC, while astronauts were more often diagnosed with cortical cataracts. In line with the latter, the NASA Study of Cataract in Astronauts (NASCA) reported an increase in both median and variability of cortical cataract compared to non-exposed astronauts, and identified a relationship between cumulative galactic cosmic radiation dose and cortical cataract [217, 218]. Confirming to Mark Little's review recognizing the absence of association between nuclear cataracts and IR [2], these observations support the concept that the lens cortex is more vulnerable to IR damage compared to the nucleus. Also, IR exposure of ground-based workers, even at low dose, correlates with pre-senile cataractogenesis as compared with age-matched controls. For instance, a Polish interventional cardiologist cohort reported a range of 0.4–1.55 Gy [219], whereas retrospective studies on radiologic technologists in the US estimated a mean dose of 77 mGy in a cohort of 109,300 in 2014 [220] and more recently in 2018, the mean cumulative estimated 5-year lagged eye-lens absorbed dose associated with self-reported cataract was 55.7 mGy [221]. Dose exposure calculated from self-reported workloads shows a wider range, with a cardiology conference cohort presenting a range of 0.1–18.9 Gy [222]. The reported incidence of lens opacities in exposed medical personnel varies both in the intensity and region of the lens affected. PSCs have been reported in 17% of the exposed O'CLOC cohort as opposed to 5% of controls [223] and in 50% of cardiologists and 41% of nurses and technicians in comparison with <10% of age-matched controls in a cardiology congress cohort [222]. A follow up study of 13,902 childhood cancer survivors who received radiation therapy as part of their treatment showed a strong correlation between 0.5 and 1.5 Gy dose exposures and the development of pre-senile cataracts later in life [224]. Therefore exposure to IR increases the risk of PSC [225, 226], but other ARC phenotypes including cortical and nuclear cataract [3, 4, 6, 7, 119, 120, 210, 227] are also observed.

Based on these findings, ICRP revised previous guidance [228] and suggested a nominal threshold of 0.5 Gy for cataract formation independent of the rate of dose delivery [29]. Current ICRP recommendations are for a reduced occupational lens dose limit of 20 mGy $year^{-1}$ (averaged over 5 years with no single year >50 mGy), highlighting the importance of determining in detail the biological response of the eye lens when exposed to low dose IR given its importance to present and future ICRP recommendations. Moreover, the US National Council on Radiation Protection and Measurements (NCRP) notes the urgency for lens-specific dosimetry and that until those technical challenges are optimised, lens-exposure should be regarded similar to whole body exposure [229-231].

4.3 Lower doses are correlated with a longer latency period

The mechanisms underlying IR-induced cataract are not fully understood and more research is ongoing to refine the proposed nominal threshold of 0.5 Gy [29]. In considering the lens cataract phenotype, the key variables that will influence the timing and phenotype of the IR-induced cataract will be the IR dose administered and the age of the individual. Early studies investigated acute IR doses (10's of Gy) that induced cataract within weeks or months in both animals [135, 232] and humans [3, 208, 209]. These early studies also identified that the age of animal at the time of exposure influenced the latency of the acute phenotype [232] and this was later interpreted as further evidence that the lens epithelium, and specifically the proliferating cells at the lens equator were the primary target for acute IR exposure [11]. As the latency for development of some cataracts in A-bomb survivors exceeded even half century [4], reassessment of the relation between IR exposure, aging and the PSC phenotype will provide us with a deeper insight of the biological mechanisms underlying low dose IR induced cataracts.

5 EFFECTS OF IR ON THE LENS

Details of the biochemical and cell biological changes in IR-irradiated human lenses are sparse (Table 1) and limited to gross phenotypic changes, such as vacuoles and cataracts formed in the posterior subcapsular region [3, 26, 207-209] due to the disruptive nature of corrective surgery and the availability of human tissue. In the following section we discuss IR-induced damages and which processes have been shown to manifest in lens cells.

	Aging	IR > 0.5 Gy	IR < 0.5 Gy
Gene expression	✔	✔	✔
DNA repair	✔	✔	✔
Cell density	✔	✔	✔
Proliferation	✔	✔	✔
Integrity of meridional rows	✔	✔	ND
Deamidation	✔	✔	ND
Racemisation	✔	✔	ND
Truncation	✔	✔	ND
Methionine oxidation	✔	✔	ND
Increased disulphide bonds	✔	✔	ND
Increased sphingomyelin and dihydrosphingomyelin	✔	ND	ND

Decreased glycerolipids	✔	ND	ND
Advanced glycation end products	✔	ND	ND
Kynurenines	✔	ND	ND
Cholesterol oxidation	✔	ND	ND
Increased cholesterol	✔	ND	ND
Cholesterol domain formation	✔	ND	ND

Table 1: **Overview of the biochemical and cell biological effects of aging and IR exposure on the lens and lens cells.** The biological mechanisms underlying ionizing radiation (IR) - induced damage to the eye lens are not completely understood. A survey of the literature shows that aging and IR > 0.5 Gy cause similar damage to the eye lens, while our knowledge of the effects of IR < 0.5 Gy is quite limited. Future research will elucidate whether the aging and IR > 0.5 Gy and < 0.5 Gy have similar effects on the eye lens, and therefore whether IR leads to accelerated aging. ND: not determined

5.1 DNA damage induced by IR exposure

IR induces direct DNA damage by generating physical breaks in DNA structures, and indirect damage through interaction with molecules leading to the formation of free radicals [233]. Direct and indirect damage include the production of single strand breaks (SSBs) and DSBs in DNA. IR-induced SSBs can be efficiently repaired [234]. In contrast, DSBs have been shown to be the main source of IR-induced mutations [235].

Genotoxicity has been observed in non-irradiated portions of the body resulting from IR exposure of other areas. First reported in 1954, where irradiation between 7.36 and 28.41 Gy directed to the spleen produced damage in bone marrow cells [236], this phenomenon is known as the abscopal effect [237, 238]. It is caused by irradiated tissue releasing molecules, e.g. ROS, that are transferred to other non-irradiated tissues, where they induce similar deleterious effects [239]. Though not directly demonstrated in the lens, the abscopal effect poses an interesting possibility in the overarching issue of eye health. Another, more recently described, consequence of indirect DNA damage is radiation-induced bystander effect (RIBE) wherein non-irradiated cells show similar features as irradiated cells as a result of intercellular signalling [238, 240]. The mechanisms underlying RIBE in other organs, e.g. transport of free radicals and ROS via gap junctions, are processes that also take place in the eye lens [241], which suggests that the occurrence of RIBE in the eye lens is possible [242].

Genomic instability is another downstream effect of IR. During proliferation of cells containing IR-induced mutations, these DNA alterations can be transferred to their daughter cells. Signals leading to production of intracellular ROS is transmitted to daughter cells, where they can cause additional DNA damage [243].

5.2 IR induced damage in the lens epithelium – Aberrant proliferation and differentiation

Our mechanistic understanding of IR-induced cataract is largely informed by animal models, but collectively with human studies they evidence a significant role for the lens epithelium in IR-induced cataract [11, 244]. High dose IR (15 Gy) caused a decrease in cell density in the germinative zone of rabbit lenses in the short term followed by a proliferative burst before returning to a pre-exposure baseline several weeks later [135]. Repressing LEC proliferation prior to IR-exposure (6 Gy/min) produced resistance to damage in a frog model suggesting cell proliferation was a key process involved in IR-induced cataract [244]. *In vitro* experiments with HLEC1 cell line showed that irradiation with ≥2 Gy (but not at <2 Gy) stimulates the proliferation of human LECs [245]. Besides aberrant cell proliferation and epithelial organization, epithelial differentiation was also changed after IR exposure (2–20 Gy; [11, 246]). These experiments were all performed with high-dose exposures. Markiewicz and colleagues performed *in vitro* and *in vivo* irradiation experiments, with the FHL124 cell line and mice respectively and included besides high dose also low dose exposures (20 mGy – 2 Gy; [247]); dose dependency in DSBs as phosphorylated histone H2AX (γH2AX) foci, increased proliferation and cell density at doses <0.5 Gy were still observed [247]. Changes in proliferation and altered gene expression were seen in the HLEC1 cell line at 4 Gy IR exposures, but not at 0.5 Gy [248] questioning whether the observed cell density and proliferation changes at low dose IR require altered gene expression.

5.3 Altered transcription as a result of IR induced DNA damage and repair

Transcriptionally active genes in cancer cells have been shown to be prone to mutation [249]. Although DNA DSBs are introduced randomly into the genome after IR exposure, those genes most likely to retain mutations are transcriptionally active genes [249]. Mutation rates in exons are lower than those found in introns because of differential mismatch repair in exons and introns [250] and H3K9me3, as a marker for open, transcriptionally active chromatin regions, is associated with higher mutation rates [251]. Recent evidence suggests that DNA repair processes involve the expansion of chromatin and eviction of histones at the site of the DSBs [252], in support of differences in DNA repair efficiency between transcriptionally active and inactive chromatin regions. For the lens this would mean that genes such as the crystallins would be more susceptible [253]. The LECs at the lens periphery have been shown to repair DSBs more slowly [247] and along with the nucleated, differentiating LFCs these are the lens cells that are transcriptionally active. Moreover, crystallin gene transcription is dependent upon both p53 [254] and N-myc [255], providing another potential direct link between transcription and DNA repair [256]. Given the long latency between exposure to low dose IR and the appearance of cataract, this is a potential mechanism contributing to the low dose IR-induced phenotype.

5.4 Reduced functionality of DNA repair pathways in lens cells

Exposure of rabbit lens epithelium to UVA (180 kJ/m^2) showed that irradiation leads to SSBs [234]. The amount of SSBs was positively correlated with exposure dose. However, these did not cause opacities because 80% of the SSBs were repaired within a timeframe of 4 hours. The effect of low dose IR on the activity of DNA repair mechanisms was measured using DSB markers γH2AX and RAD51 (ranging from 20 mGy to 2 Gy; [247, 257]). γH2AX codes for an activator of DNA repair and RAD51 is part of the homologous recombination DNA repair systems. In FHL124 cells, the expression levels of these markers

showed a linear dose response to exposures at lower than 0.5 Gy. Mouse LECs exposed to 0.01 and 0.1 Gy showed an increase of γH2AX. This increase was higher in the central zone than in the germinative and transitional zones. Comparison of γH2AX levels in the LECs with those in lymphocytes showed that DNA repair in LECs is slower [247]. Based on the latter, we conclude that irradiation leads to a slower DNA repair in the LECs. In particular, DNA repair appears to vary between lens epithelium zones with the transcriptionally active germinative and transitional zones demonstrating slower and fewer repair incidents [135, 247].

5.5 Effects of irradiation on the fiber cell proteins

In order to develop and maintain lens transparency, lens proteins must not aggregate at high concentrations [258]. IR-induced oxidative stress causes aberrant protein aggregation, which leads to light scattering in the lens and eventually leading to cataract formation [259]. These IR-induced aberrant protein aggregates are formed through post-translational modifications including oxidation, deamidation, truncations and cross-linking [111]. Irradiated α-crystallins form aggregates *in vitro* [260]. Follow up studies using γ-irradiated rat lenses showed that this triggered oxidation and deamination of crystallins [261]. Exposure of α-crystallin to UV also resulted in tryptophan degradation and inactivation of their chaperone function [260]. Still, the effects of low dose irradiation on protein structure remain largely unknown [262].

5.6 Effects of irradiation on lens membrane lipids

Cataract patients have increased levels of lipid peroxidation products in comparison to age matched controls [129]. Lipid peroxidation has been shown to cause changes in the structure and permeability of membranes after exposure *in vitro* [263]. IR exposure stimulates formation of ROS which can oxidise the lipids in the eye lens. Oxidation of cholesterols generates oxysterols [264, 265]. However, the mechanisms through which low-dose IR-induced lipid oxidation of cholesterol causes lens damage are now under investigation.

6 CONCLUSION - THE LENS AS A MODEL TO STUDY THE LINK BETWEEN AGING AND LOW DOSE IR

In the lens, the aging process manifests itself first by the development of presbyopia in the fifth decade of life (Figure 2; [117, 118]) and then ultimately in the formation of ARC [10]. These are quite distinct albeit interrelated phenotypic stages [266] and consistent with the concept that ARC represents a disease associated with accelerated aging [43, 44]. Biomolecular damage accumulates during the lifetime of the individual. IR exposure is an addition to the damage accumulated due to the aging process alone (see Table 1). The lens retains lipids [17] and proteins [10] over its lifetime meaning that any IR-mediated non-DNA damage persists and is not removed. Thus, the rate of damage accrual can influence the timing of cataract during the aging process [44] as the cataractogenic threshold is reached sooner (Figure 2). For each individual, this threshold is determined by age, lifestyle, environment and genetic background i.e. all those factors known to contribute to human cataractogenesis from epidemiological studies. The damaging effects upon cells and their biomolecules through the generation of free radicals by IR exposure will be retained in the protein and lipid components in the LECs and LFCs ([10, 24, 129]; Figure 3). DNA damage, though mostly repaired, might also influence lens health via the retention of

mutations or alteration in cell metabolism [33, 51]. This IR-induced damage therefore contributes to the cataractogenic load (i.e. lens deleteriome [122]). The genetic background, metabolic, environmental and lifestyle experiences of the individual will affect both the timing and nature of their cataract (cortical or nuclear) depending on when the cataractogenic threshold is reached (Figure 2).

The lens therefore provides an experimental system to study aging processes. Incidental IR exposure can be measured and experimental doses can be controlled, which is not easy for other environmental insults such as smoking and UV-exposure when linked to cataract [191, 267, 268] and accelerated aging (e.g. [269-272]). The fact that in several cohorts, PSC caused by exposure to low dose IR follows a linear dose response relationship (rather than a threshold-type relationship) for various age groups provides an opportunity to identify the IR-specific cataractogenic load and the mechanism(s) involved. Diverse patient groups have been shown to be key to understanding disease dynamics, with the Beaver Dam Eye Study being a hallmark example of the above [7, 187, 188]. The study of low dose IR and its effect on cataractogenic load can help deliver mechanistic insight into both cataractogenesis and the radiobiological effects of IR. Understanding factors which contribute to increased rate of cataract progression could aid the delay of cataract onset over the lifetime of an individual.

7 ACKNOWLEDGMENTS:

The authors are part of the LDLensRad project that has received funding from the Euratom research and training programme 2014-2018 in the framework of the CONCERT [grant agreement No 662287]. The financial support of the Royal Society (RAQ; IE151271) the National Eye Research Foundation (AAK; SAC014) and Fight for Sight UK (RAQ, MJ; 1584/1585) are also gratefully acknowledged.

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Figure 1: The eye, the lens and the epithelium (A) The eye lens is located behind the cornea and in front of the retina. It is positioned in the eye by ligaments from the ciliary body and the hyaloid membrane by Wieger's ligament that then embed into the lens capsule. The hyaloid membrane separates the two humours of the eye, the aqueous and vitreous. **(B)** Schematic of the epithelium that covers the anterior hemisphere of the eye lens. The epithelium has different zones, the central, germinative and transitional zones. The density of the cells (blue dots) changes across the epithelium. Epithelial cell proliferation is greater in the germinative zone (dividing cells represented by red dots). Cells in this region embark on their programme of differentiation by moving into the transitional zone and then into the meridional rows where they become organized ready to form lens fiber cells. The meridional rows are highly organized, but with age this organization declines. **(C)** An example of a mouse lens stained with DAPI to identify cell nuclei in the lens epithelium. The lens is orientated in the same way as the schematic. Notice the change in cell density between the different zones and the organization in the meridional rows.

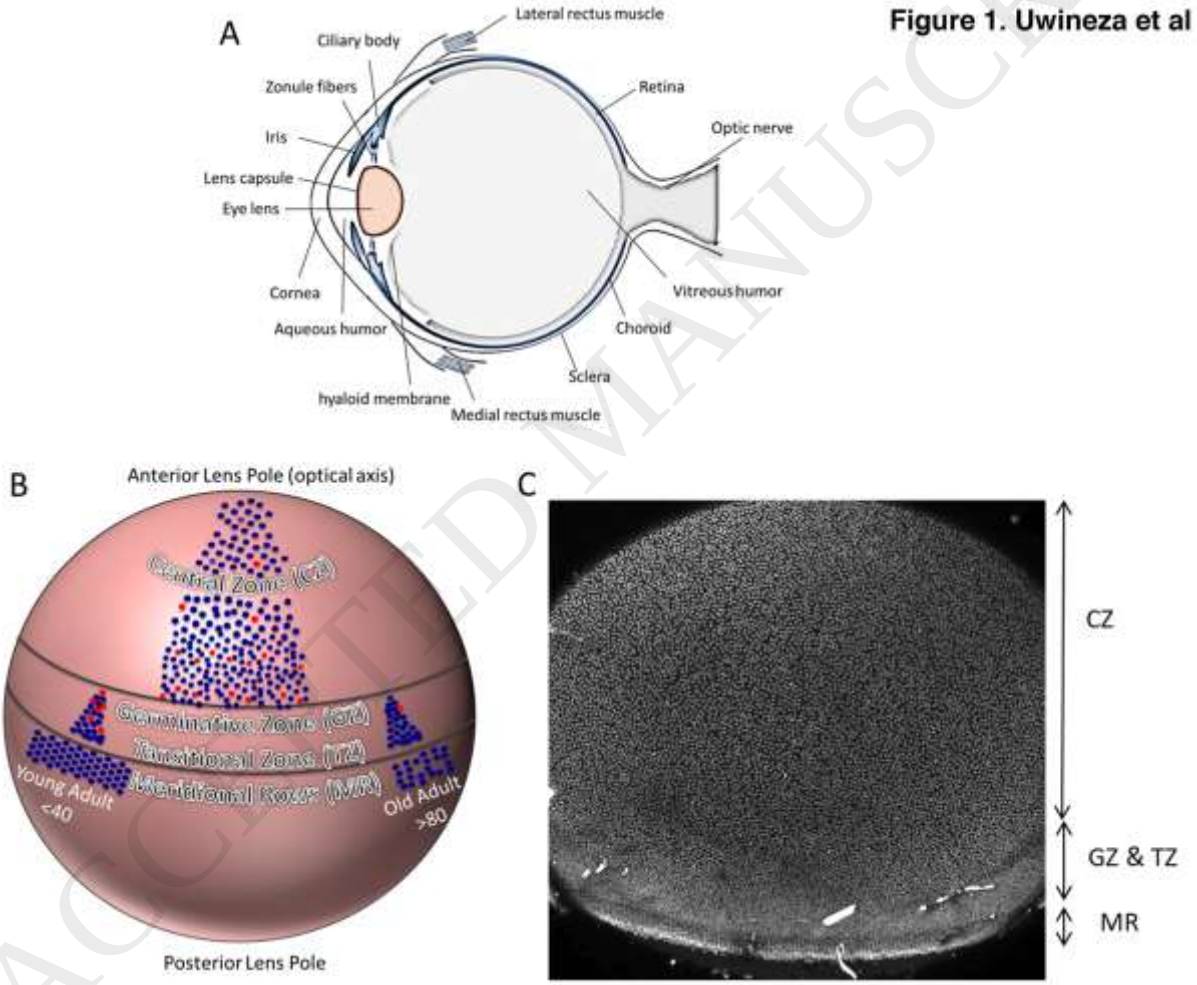

Figure 2. Lifelong cataractogenic load accumulation and the latency of cataract (A) Timeline for lens aging and the appearance of cataract under the influence of the accumulated cataractogenic load. Aging of the lens is dependent upon genetic, environmental and stochastic factors. A universal aging effect upon the lens is the development of presbyopia at the end of the fourth decade of life. The appearance of cataract depends upon the accumulated cataractogenic load, which is specific to each individual. Age related cataract (ARC) is phenotypically variable, although the most frequent ARC is a nuclear cataract. The additional burden of IR exposure can accelerate

cataractogenesis, but there can be a long latency period between the exposure and the resulting cataract. Where cataract incidence involving an IR event has been studied, these have almost entirely involved younger people (<30) and whilst PSC is a common cataract phenotype as a result of acute IR exposure, cortical and nuclear cataracts are also seen after low dose IR exposure. **(B)** The accumulated, cataractogenic load determines when cataract will appear in the lens of an individual. This cataractogenic load accumulates differently for each individual dependent upon the genetic, environmental and stochastic factors. Simplistically this is represented as a threshold, but for a population this threshold is reached at different ages as reflected in wide age range of cataract onset. **(C)** IR adds to the cataractogenic load and therefore for the individual represented in (B), the threshold for cataract formation is reached earlier because the rate has increased.

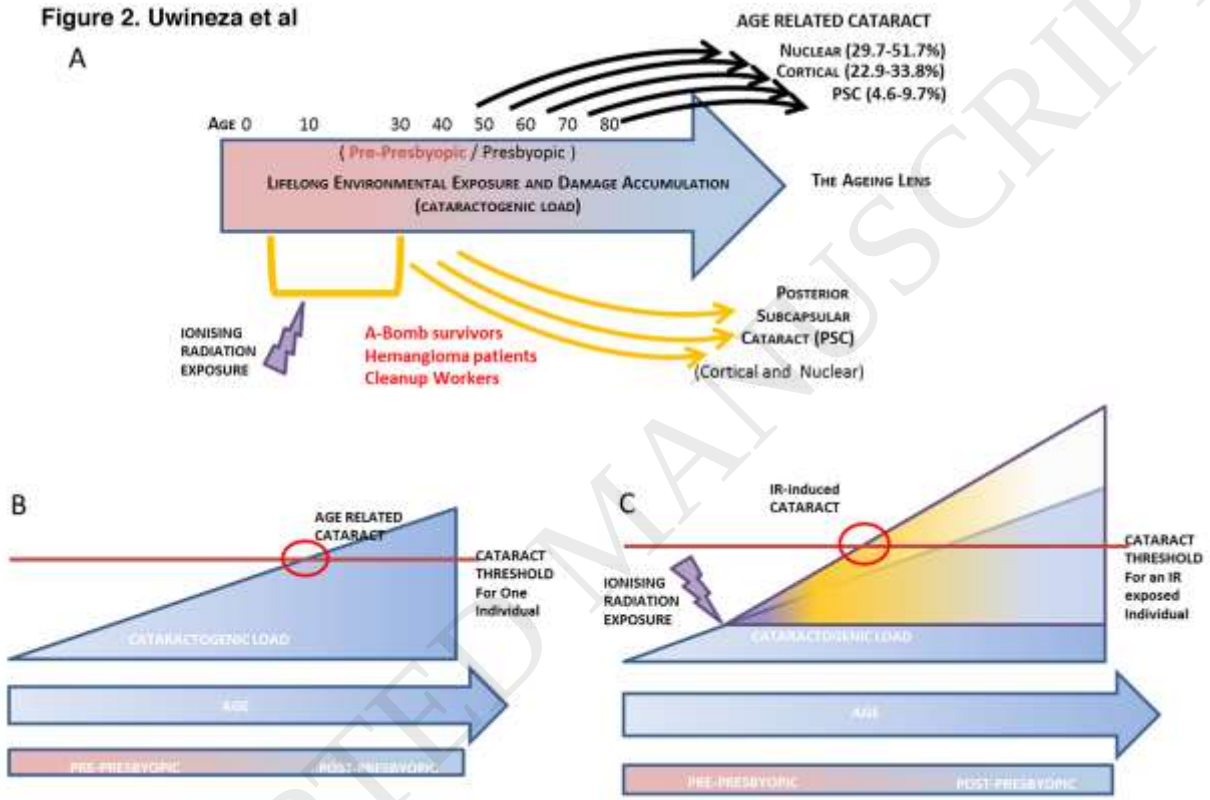

Figure 3: IR induced damage to the genome, proteome and lipidome of lens cells. The two cell types in the lens, Lens Epithelial Cells (LECs) and Lens Fiber Cells (LFCs), are both sensitive to ionizing radiation (IR). IR can cause the production of Reactive Oxygen Species (ROS) from water molecules. ROS causes double stranded breaks (DSBs) in the DNA within LECs and LFCs. ROS can also trigger protein modifications that lead to protein aggregation and the oxidation of lipids in the membranes of both LECs and LFCs.

Figure 3. Uwineza et al