**Ionizing radiation induced cataracts: Recent biological and mechanistic developments and perspectives for future research**

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**Abstract**

The lens of the eye has long been considered as a radiosensitive tissue, but recent research has suggested that the radiosensitivity is even greater than previously thought. The recent recommendations of the International Commission on Radiological Protection (ICRP) to substantially reduce the annual occupational equivalent dose limit for the ocular lens have now been adopted in the European Union and are under consideration around the rest of the world. However, ICRP clearly states that the recommendations are chiefly based on epidemiological evidence because there are a very small number of studies that provide explicit biological, mechanistic evidence at doses <2 Gy. This paper aims to present a review of recently published information on the biological and mechanistic aspects of cataracts induced by exposure to ionizing radiation (IR). The data were compiled by assessing the pertinent literature in several distinct areas which contribute to the understanding of IR induced cataracts, information regarding lens biology and general processes of cataractogenesis. Results from cellular and tissue level studies and animal models, and relevant human studies, were examined. The main focus was the biological effect of low linear energy transfer IR, but dosimetry issues and a number of other confounding factors were also considered. The results of this review clearly highlight a number of gaps in current knowledge. Overall, while there have been a number of recent advances in understanding, it remains unknown exactly how IR exposure contributes to opacification. A fuller understanding of how exposure to relatively low doses of IR promotes induction and/or progression of IR-induced cataracts will have important implications for prevention and treatment of this disease, as well as for the field of radiation protection.

*Keywords:*

IR

Radiation cataract

Radiation lens effects

Lens biology

Cataract mechanisms

Dosimetric modeling

*Abbreviations:* A-bomb, atomic bomb; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; BER, base excision repair; Ca2+, calcium ions; CDKN1A, cyclin-dependent kinase inhibitor 1A; CHO, Chinese hamster ovary; COX-2, cyclooxygenase 2; CT, computed tomography; Cx, connexin; DSB, DNA double strand break; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; EPHA2, ephrin receptor tyrosine kinase type A2; EU, European Union; FGF2, fibroblast growth factor 2; GJ, gap junction; GZ, germinative zone; hES, human embryonic stem; ICRP, International Commission on Radiological Protection; ICRU, International Commission on Radiation Measurements and Units; IκB, inhibitor of nuclear factor κB; IKK, inhibitor of nuclear factor κB kinase; IL, interleukin; IR, ionizing radiation; LEC, lens epithelial cell; LET, linear energy transfer; LFC, lens fiber cell; Lim2, lens intrinsic membrane protein 2; LNT, linear-no-threshold; MCNP, Monte Carlo N-particle; MIRD, Medical Internal Radiation Dose; MMP, matrix metalloproteinase; MR, meridional row; NBN, nibrin; NBS, Nijmegen breakage syndrome; NDRG2, N-Myc downstream-regulated gene 2; NER, nucleotide excision repair; NF-κB, nuclear factor κB; NTE, non-targeted effect; OFZ, organelle free zone; OGG1, 8-oxoguanine DNA glycosylase 1; PI3K, phosphatidylinositol 3-kinase; Prdx6, peroxiredoxin 6, PSC, posterior subcapsular cataract; Ptch1, patched 1; R, Röntgen; rep, Röntgen equivalent physical; RIBE, radiation induced bystander effect; RIGI, radiation induced genomic instability; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; SSB, DNA single strand break; TGFβ, transforming growth factor β; Tm, tropomyosin; TNFα, tumor necrosis factor α; TZ, transitional zone; UV, ultraviolet; XPD, xeroderma pigmentosum D; XRCC1, X-ray repair complementing defective repair in Chinese Hamster cells 1; γH2AX, histone H2AX phosphorylated on serine 139; 53BP1, p53-binding protein 1.

**1. Introduction**

 The lens of the eye has long been recognized as being highly responsive to ionizing radiation (IR) – the lens is one of the most radiosensitive tissues among the ocular structures, and, indeed one of the most radiosensitive tissues in the body [1,2]. IR exposure can lead to development of cataracts, defined clinically as progressive opaqueness of the lens leading to loss of vision (and, in this work, according to the original definitions in each paper referenced). Although cataractogenesis is a complex, lengthy process, following the epidemiological observations, it is assumed that IR exposure chiefly leads to the formation of posterior subcapsular cataracts (PSCs; e.g. [3]). PSCs are distinct from age-related or congenital, hereditary cataract phenotypes that typically form at the lens nucleus (e.g. [4]). Note, though, that PSCs have also been associated with other factors including aging [5].

 In radiation protection terminology, IR effects are generally classified as either “deterministic effects”/”tissue reactions” (effects which only occur above a minimum threshold dose of IR) or “stochastic effects” such as cancer and heritable effects, the risk of which increases with any dose, no matter how small, according to the linear-no-threshold (LNT) model [6]. Cataracts are classified for radiation protection purposes as deterministic effects. Until very recently, chiefly based on the work of Merriam and colleagues from the 1950s and 1960s [7,8], the threshold for detectable opacities was assumed to be on the order of 2 Gy for acute exposures and 5 Gy for highly fractionated or protracted, chronic, exposures. For vision impairing cataracts, these thresholds were 5 Gy and >8 Gy, respectively.

 However, a number of recent epidemiological studies and reanalyses of the datasets have demonstrated that acute doses of low linear energy transfer (LET; the average amount of energy deposited per unit track length [9,10]) IR on the order of 1 Gy can lead to cataract formation [3,11]. A detailed review of this subject led the International Commission on Radiological Protection (ICRP) to propose a nominal threshold (definedat the 1% incidence level) of 0.5 Gy for cataract induction irrespective of the rate of dose delivery [12]. No distinction was made between acute and chronic exposures. Further, ICRP recommendations for a reduced occupational lens dose limit of 20 mSv per year(over an average of 5 years with no single year >50 mSv) have now been implemented within European Union (EU) law, and it is likely that similar reductions will be applied in many other nations worldwide.

 In reviewing the literature on IR induced cataracts, ICRP concluded in 2011 that “there is no direct evidence that a single damaged progenitor lens epithelial cell (LEC) can produce a cataract.” Hence, although this is a somewhat simplified interpretation, IR induced cataract is still considered a deterministic effect or tissue reaction [12]. ICRP Publication 118 does, however, indicate the need for future research which “may elucidate the true mechanism of cataract formation” [12]. Without a full understanding of the mechanisms behind the processes of IR cataractogenesis, the recommended threshold of 0.5 Gy may reflect an over- or underestimation of the “true threshold”. Indeed, there is evidence in the literature to dispute the deterministic classification. Data from several recent mechanistic studies (discussed in sections 3 and 4 of this manuscript) and some epidemiological studies (discussed in section 5) suggest that effects to the lens may be caused by processes other than involving cell death that would be typical of a traditional deterministic effect.

 For instance, LECs in the germinative zones (GZs) at the periphery of the lens epithelium (Fig. 1) have been shown to be critical to IR induced cataractogenesis. Low dose IR induces DNA damage and increases LEC proliferation [13]. Meanwhile, high dose IR decreases cell density in GZ and disrupts cell organization in the GZ and meridional rows (MRs) [14]. Further, the oxidative stress pathway is suspected to play a major role in the development of cataract, for which the genetic basis remains unclear [15]. The above studies also suggest that the late onset of cataracts associated with IR may be, at least in some cases, due to loss of antioxidant capacity in the lens which might, in turn, be related to the aging process that determines the decline or otherwise of lens function [16,17]. This could indicate that acceleration of the aging processes is one mechanism of IR cataractogenesis [18]. However, in consideration of all available evidence to date, it is important to note that much of the mechanistic research conducted in this field has focused on use of animal models to yield mechanistic details of IR responses (e.g. [19]; to be discussed in detail in section 4). It is much harder to use such models to monitor cellular/molecular responses to IR with time and follow the process of cataract formation in full, thus there are still many unanswered questions.

 This review aims to bring together the current information regarding mechanisms of IR cataract initiation and development. The review begins by considering relevant information from the general fields of lens biology and cataractogenic processes in section 2, as there are likely to be many commonalities between IR and other types of cataracts. Recent studies on IR cataractogenesis are reviewed in detail in the following sections, classified by the type of the study: *in vitro* cellular and tissue studies (section 3); animal models (section 4); and information from epidemiological and human studies (section 5). IR type and exposure conditions clearly also affect the induction process; the relevant dosimetry aspects are thus reviewed in section 6. Cataract is a highly multifactorial disease, and the importance/impact of other modifying factors for IR induced cataracts is therefore reviewed in section 7. Finally, the data are interpreted to produce an up-to-date summary of the available information, in order to draw conclusions regarding the current status of knowledge and identify gaps for further research. This paper focuses chiefly on the biological effects of low LET IR to the ocular lens; the companion papers in this issue will further deal with the findings in relation to the biological effects of high LET IR [20] and human IR induced cataracts [21].

**2. Lens biology and cataractogenesis**

*2.1. Structure and function*

 The ability to detect light, respond to a stimulus and elicit behavioral responses is common to many forms of life [22]. Light is necessary to our physiology, setting the daily rhythms of life though circadian responses [23–25]. Light is also, of course, key to vision. The retina is the detection system for the light that is focused onto the optic nerve through the refractive properties of the transparent cornea and lens.

 The human lens starts to form by weeks 4-5 post fertilization (Carnegie Stage 15) [26]. The secondary lens fiber cells (LFCs) are formed by Carnegie stage 21 and, from this point, the lens grows in size throughout life [27]. The lens is located in the anterior hemisphere of the eye, behind the cornea and iris, and is surrounded by the aqueous humor (anterior) and vitreous body (posterior) [28]. The lens is both transparent and elastic to allow focusing of near and far objects onto the retina in order to produce a well-defined image. During embryogenesis, the lens is filled by LFCs with a layer of LECs lining the anterior internal surface of the lens. LECs undergo division in the GZ, from which the daughter cells migrate and differentiate into LFCs free from cell nuclei and other cellular organelles, which are broken down in the differentiation process. The transparency of the lens is assumed to rely on the lack of organelles within the LFCs as well as the highly structured organization of the proteins (crystallins) and low water content. Fig. 1 illustrates protein fiber and cellular organization within the lens.

 The mature lens is an avascular and transparent tissue contained within its own basement membrane called the lens capsule [29]. The lens capsule defines the lens perimeter, which nutrients, growth factors and antioxidants must cross to enter the lens. These are two very distinctive features for living tissue, requiring specific cellular structural organization for lens function [30], which must allow for the continuous growth of the lens throughout life whilst also maintaining its transparency and refractive properties. When changes to these processes occur, the transparency and optical properties of the lens can be altered, compromising its function [30–32]. It is also important to note that tumorigenesis does not occur in the human lens (e.g. [33]).

<Figure 1 here>

*2.2. Physiology of the lens*

 Lens metabolic activity is restricted to the lens epithelium and younger nucleated LFCs surrounding the organelle free zone (OFZ), which is also responsible for 90% of the oxygen consumption [34,35]. This superficial cortex is responsible for supporting the metabolism of the whole lens, and the lens epithelium indeed provides 30% of the required adenosine triphosphate (ATP, which facilitates energy transport within cells) [36,37]. DNA transcription and protein synthesis are restricted to the outer cortex where LFCs still possess the necessary organelles. The majority of mature LFCs, including those at the lens nucleus, no longer actively support these ATP-sapping activities [38–41]. Furthermore, metabolism in the nuclear LFCs is minimal [42–45], defining another physiological metabolic state that is significantly depressed. An active circulation system connecting the lens cortex to the lens nucleus carries ions and metabolites to supply and sustain the mature LFCs with nutrients and also controls the volume of these cells [46].

 It has been proposed that cytoplasmic sodium and potassium concentrations within the lens are maintained by Na, K-adenosine triphosphatase (ATPase), which actively extrudes sodium and imports potassium [47]. Indeed, Donaldson et al. [46] propose that the surface cells (including LECs and young LFCs) contain Na+-K+ pumps and K+ channels, which together generate a negative electromotive potential. The permeability of the mature LFCs is dominated by Na+ and Cl– leak conductance [48]. In these inner cells, a negative membrane potential is maintained by connection to the surface cells via connexin (Cx) mediated gap junctions (GJs). Thus, LFCs and surface cell membranes need significant water permeability. If ATPases change over time, for example due to aging or by oxidative processes, an osmotic imbalance happens leading to the formation of vacuoles by the incoming water (“osmotic cataracts”) [49]. LFCs also need a mechanism to obtain glucose and the equatorial surface cells export the flux of Na+ that is arriving from the inner LFCs [50]. These influx and effluxes are essential to maintain the homeostasis and transparency of the lens.

 Another crucial factor for lens transparency is oxygen itself. Oxygen diffuses from the surrounding vitreous humors into the lens [51]. In humans, the oxygen level is approximately 14 mm Hg near the anterior lens epithelium [52], but below 8 mm Hg at the posterior of the lens [53]. Compared to other tissues, the lens is relatively well protected from oxidative damage because of low oxidative metabolism [34,35]. It is thought that the high consumption of oxygen by the lens epithelium and superficial cortex maintains the LFC compartment at a low oxygen tension, protecting the lens proteins, although the lens has also been described as particularly vulnerable to oxidative stress [54].

 The lens epithelium has also evolved many protective mechanisms to prevent oxidative damage [34]. These range from simple scavenger systems, free-metal binding proteins, to more advanced enzyme protective systems (glutathione peroxidase, catalase and superoxide dismutase) including chaperone proteins (crystallins) to prevent aggregation of oxidized proteins within the cell [55,56].

 The low oxygen tension may suggest that generated free radicals react directly with molecules such as DNA, proteins or membrane lipids and thus damage LFCs [57]. Nuclear LFCs lose the ability to synthesize proteins, carry out oxidative metabolism and synthesize new membranes [51]. Also, while lens cells can die and thus there is some cell turnover, all lens cells remain in the lens throughout life; hence the lens accumulates damage throughout life [32]. In addition, there is limited turnover of proteins [40,41] and lipids [58], so free radical damage will lead to polymerization and cross-linking of these, resulting in an increase in the water insoluble protein content, aggregation and opacification [59].

 Exposure to charged particles can alter the expression of matrix metalloproteinases (MMPs) [60], which are the enzymes responsible for remodeling the extracellular matrix (ECM) and for releasing the survival, proliferation and differentiation factors [32,61] for LECs [62]. IR can also alter the ECM itself [63,64], as further discussed in section 3.2.

 Lens cells contain relatively high concentrations of antioxidants including ascorbate [65] and glutathione [66]. Glutathione prevents free radical damage to lenticular biomolecules, by reacting directly with free radicals, products from photon damaged tryptophan as well as regenerating ascorbate [67]. To maintain glutathione levels, the pentose phosphate pathway assists through the production of NADPH which reduces the oxidized glutathione [67]. Despite the importance of glutathione to lens homeostasis, the source of this antioxidant is still unclear [68], but the presence of glutamate and cysteine transporters in LFCs could mean that the source is LFCs themselves [69].

 The lens plasma membranes also provide antioxidant protection. The nuclear LFC membranes are less permeable to oxygen than those in the lens cortex and, as the lens ages, its permeability to oxygen decreases even further [70,71]. In addition, the unique lipid composition of the human lens nuclear LFC membrane also helps protect against free radical mediated oxidation. Sphingomyelin and dihydro-sphingomyelin both help protect cholesterol from oxidation [72], but the signs of cholesterol oxidation remain apparent during aging [73] and there are characteristic cholesterol oxides that typify human cataract [74].

 A circulation current contributes to transport and the renewal of soluble proteins in the LFC compartment [40,41], but with age the cytoplasm of the mature LFCs becomes stiffer [51]. This slows the diffusion from the surface cells to the core of the lens and decreases the removal of waste products. For instance, very small molecules such as H2O2 and the superoxide anion can diffuse to the lens nucleus; however, glutathione produced by epithelial cell layer and younger LFCs cannot diffuse past the barrier, which develops in the fifth decade [75]. These changes make the core of the lens more susceptible to oxidative damage and therefore to the consequences of IR damage. However, this could be interpreted to indicate that nuclear cataracts would be the most commonly observed type of IR induced cataract, which is in contrast to most of the evidence to date [3,11,12]. The reasons for this are unclear; however, a possible explanation is the length of the human lifespan – it has been proposed that if humans lived longer, then increased risks for nuclear cataracts may begin to be detected following IR exposure [76].

 DNA repair pathways are also involved in maintaining the transparency of the lens and altered expression of DNA repair genes is associated with age-related cataract. The lens epithelium expresses at least 92 genes involved with DNA repair. Those genes are required to ensure the integrity of the cellular genome, including nucleotide excision repair (NER), base excision repair (BER), DNA strand break repair, and direct reversal of DNA damage [77].

*2.3. Lens fiber cell differentiation*

 LFC differentiation requires a complex and coordinated chain of biochemical and morphological events [31]. After LECs initiate the differentiation program, adopting the hexagonal cell profile, they migrate to form the LFC compartment of the lens. The first characteristic morphological change involves a dramatic elongation of the cells. This requires reorganization of the cytoskeletal machinery and particularly intermediate filaments [78]. This is accompanied by the expression of an extensive range of LFC specific proteins including crystallins [79,80] and membrane associated proteins (e.g., MIP [81] and MP20 [82]), all of which help establish the cell environment to provide the transparent and refractive properties needed to refract light onto the retina. The final step of LFC maturation requires the programed removal of organelles (nucleus, Golgi apparatus, endoplasmic reticulum and mitochondria [31,83–85]), which establishes lens transparency and leads to the formation of an OFZ [84]. This process is carried out in a rapid and coordinated manner within the space of a few cell layers. The result of this last event is a complete, fully differentiated LFC. Disturbances of this process (i.e. incomplete removal of organelles) causes light scattering in the lens and indicate changes in the lens differentiation program including alterations of the expression of proteins, typically found in the mature LFCs (e.g. crystallins), as discussed in the following sections.

 Formation of a mature LFC, however, is not an endpoint, as age-related changes continue to alter the solubility and complexity of the biomolecular signature of LFCs because the eye lens is a closed system, which retains all theLFCs ever formed (even those that helped close the lens vesicle during the earliest stages of the formation of the lens during embryogenesis). Proteins and other cellular components are subject to oxidation, glycation and deamidation with little prospect for repair or replacement of damaged components [41]. The mature LECs provide metabolic support to the entire lens [36], where repair, regeneration and transport systems are also concentrated and most active. The lens epithelium is therefore essential for maintaining lens homeostasis [36,37].

*2.4. Opacification*

 Cataract is a disease characterized by progressive clouding of the lens, eventually resulting in visual impairment. Cataract has been recorded and treated since Egyptian times, although the technique has been finessed from the original approach of removal with a sharp stick to careful, precise and successful surgical intervention replacing the clouded lens with an artificial plastic replacement. Cataracts tend to be classified according to their location and/or severity (see section 4.1) within the lens.

 Cataract formation is an iconic age-related disease, with the majority of cataracts being associated with lens nucleus [75]. Cataractogenic processes are complex and, as discussed later on in this review, involve several different mechanistic strands and clinical endpoints, for which the general processes have been reviewed in detail [29]. In brief, nuclear cataracts occur on the lens nucleus; cortical opacities begin at the lens cortex and then form characteristic “spokes” towards the center of the lens, and PSCs form on the capsule at the back of the lens. The phenotype of a nuclear cataract is a hardened, opaque nucleus which is often brown in color. Non-enzymatic, post translational modifications and the accumulation of fluorescent chromophores with aging increase susceptibility to oxidation and cross-linking and thus scattering of light [75]. Age-related modifications also lead to the breakdown of intracellular antioxidant transportation, so that a glutathione barrier forms around the lens in or around the fourth or fifth decade of the human life [5]. Cortical cataracts are associated with mutations and epithelial changes, particularly following ultraviolet (UV) and IR exposure. Such cataracts arise due to changes in membrane permeability and enzyme function and shear-stress damage to lens fibers with continued accommodative effort [5], oxidative stress and associated processes (e.g. telomeric shortening) and/or lipid peroxidation [5,87–89]. Increased levels of calcium, leading to osmotic stress, for example, are also thought to play a role [90]. Aberrant growth of the epithelium is the basis of some forms of cataract [91], leading to persistence of organelles in LFCs [92], and apoptosis signaling and proteolytic pathways have been implicated in LFC differentiation [31].

Cataracts were one of the first pathologies identified with a genetic component through blood group association. Today, ~1000 mutations in genes associated with cataract are listed in an online database [86; <http://cat-map.wustl.edu/>]. Many genes thus affect lens and cataract formation, including through pleiotropic action [93]. Cataract has been linked to race with those of Asian [94] or African American [95] descent, for example, showing an increased prevalence and earlier onset of cataract. Other lifestyle factors have been associated with cataract formation such as vegetarianism [96]. In addition, a number of health conditions (e.g. diabetes) lead to increased risk of formation of cataracts [88]. A large number of factors known and/or suspected to be involved in IR cataractogenesis are discussed in detail in sections 6 and 7.

**3. Evidence from cellular and tissue level, *in vitro*/*ex vivo,* studies**

 Current knowledge considers genomic damage of LECs to be one of the key mechanisms for initiation of IR cataractogenesis [97]. This fits with the well-known genotoxic nature of IR with downstream effects including cell division, transcription and LEC differentiation into LFCs [98]. However, a number of studies suggest alternative mechanisms, for example, those due to individual genetic status [86,93]. Indeed, following IR exposure, a number of competing mechanistic strands may act in parallel or in cooperation to produce opacities.

 *In vitro* studies (summarized in Table 1) have yielded information on both genotoxic stress induced by IR and the associated oxidative stress. This may result in aberrant cell division, cell migration and differentiation potentially leading to cataract *in vivo* [31,99,100]. However, *in vitro* studies also suggest that lens cells are able to modify their microenvironment, further promoting aberrant behavior such as the expression of transforming growth factor β (TGFβ) and fibroblast growth factor 2 (FGF2) which may promote migration (e.g. [101]), and release of MMPs [62]. Other *in vitro* studies have investigated oxidative stress without IR exposure, and also noted the change in expression of certain proteins that could be involved in premature senescence, potentially inhibiting successful differentiation into LFCs (e.g. [102]). The relevant literature is reviewed in detail in this section.

Table 1. *In vitro* studies with LECs (primary cells/cell lines) which have identified processes that may be involved in IR induced cataract development.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| References | IR | Cell origin | Materials | Contribution to mechanistic understanding |
| Chang et al., 2000 [103] | Y | Human | LECs | Rapid and transient increase in FGF2 following high LET IR. |
| Chang et al., 2005 [104] | Y | Human | LECs | Transcription and translation of *CDKN1A* are both temporally regulated after exposure. LET effects likely play a role in the misregulation of gene function in these cells. |
| Fujii et al., 2006 [105] | Y | N.A. | Crystallins  | Irradiation of protein showed structural changes such as increases in hydrophobicity and larger than usual aggregates. |
| Chang et al., 2007 [60] | Y | Human | LECs (various) | IR induced alterations in the misregulation of MMP expression, which may impact selective ECM remodeling and cell differentiation. |
| Fujimichi and Hamada, 2014 [106] | Y | Human | HLEpiC (ScienCell) | Inactivation of majority of cells following exposure whilst those that persisted showed excessive growth. |
| Markiewicz et al., 2015 [107] | Y | Human /Mouse | FHL124 / Mouse LEC | Linear dose response curves for γH2AX and RAD51 DNA damage markers; slower DNA repair response of the lens proliferating region compared to the central region. |
| Blakely et al., 2000 [108] | N | Human | 18-week prenatal lenses | With time in culture, the cells demonstrate morphologic characteristics of, and express protein markers for, LFC differentiation. |
| Kubo et al., 2009 [76] | N | Mouse | Isolated LECs (8 weeks) | Cells deficient for *Prdx6* lost control of homeostatic levels of ROS and as a result increased expression of proteins like TGFβ, subsequently inducing EMT. |
| Zhang et al., 2011 [102] | N | Human | SRA01/04 | Upregulation of *NDRG2* induces cell morphological changes, reduces cell viability, and especially lowers cellular resistance to oxidative stress. |
| Kayastha et al., 2014 [101] | N | Human | FHL124 | Supplementation with TGFβ and FGF2 induced cell migration and proliferation likely through the PI3K/Akt pathway. |
| Bannik et al., 2013 [110] | Y | Mouse | C57BL/6J | LECs in this mouse strain show a steeper dose response than in lymphocytes when comparing γH2AX foci. The repair of foci was slower in lymphocytes. |
| Wang et al., 2005 [111]  | N | Human | FHL124 | Intact endoplasmic reticulum Ca2+ stories required for lens cell survival and growth. |

C57BL/6J, inbred laboratory mouse strain; CDKN1A, cyclin-dependent kinase inhibitor 1A; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; FHL124, spontaneously immortalized human LEC line [112]; FGF2, fibroblast growth factor 2; γH2AX, histone H2AX phosphorylated on serine 139; HLEpiC, primary human lens epithelial cell; IR, ionizing radiation; LEC, lens epithelial cell; LET, linear energy transfer; LFC, lens fiber cell; MMP, matrix metalloproteinase; N, without ionizing irradiation; N.A., not applicable; NDRG2, N-Myc downstream-regulated gene 2; PI3K, phosphatidylinositol 3-kinase; PI3K/Akt pathway, signal transduction pathway that promotes survival and growth in response to extracellular signals; RAD51, gene coding for proteins in the DNA repair pathway; ROS, reactive oxygen species; TGFβ, transforming growth factor β; Y, with ionizing irradiation.

*3.1. DNA damage*

 In common with many other tissues, LEC DNA damage from natural or environmental insults can generally result in the formation of DNA double strand breaks (DSBs), which need to be repaired by the cell. The majority are repaired quickly, but there is a chance of misrepair or persistence of unrepaired damage. This was recently demonstrated in the mouse lens epithelium [107], using IR to induce DNA DSB and analyzing the resulting repair kinetics using immunostaining of foci. Repair appeared slower in the peripheral region of the monolayer when compared to peripheral lymphocytes following exposure to 20 mGy or 100 mGy of X-rays. The spontaneously immortalized human LEC cell line FHL124 [112] was also shown to exhibit a dose response in DNA damage measured by histone H2AX phosphorylated on serine 139 (γH2AX) and RAD51 foci; the effect could clearly be at doses as low as 280 mGy [107]. Clonogenic cell survival has also shown a clear dose response, albeit in a different primary cell line [106]. Mouse LECs have also been shown to be more sensitive to IR induced DNA damage than lymphocytes [107,110], and it has also been suggested that the peripheral region is more sensitive than the central region of the lens epithelium [107].

 Although not the subject of this review, the repair kinetics of lens cells following UV exposure has also been investigated, and this may have some relevance for IR cataracts. Cyclobutane pyrimidine dimers and pyrimidine-pyrimidone (6–4) photoproducts, typical of UV exposure, were the aberrations investigated. LECs were competent in the repair of UVB induced damage; 95% of the (6-4) photoproducts and only 50% of the cyclobutane dimers were repaired 24 h post irradiation [113]. UV response of rabbit LECs has also been investigated, revealing that UVA irradiation could induce significant levels of DNA single strand breaks (SSBs). This response was correlated with increasing UVA dose, and 80% of SSBs were repaired following a 4 h recovery period. These cells also showed typical repair kinetics with an initial fast repair phase followed by a secondary slower repair phase [114].

 Data from other studies not directly assessing the effects of IR exposure can also prove informative. For instance, when bovine LEC cell lines were treated with H2O2, the resulting oxidative stress induced extensive DNA damage within LECs [115]. The quantity of damage increased linearly with increasing H2O2 concentration, although no DSBs were observed as these are not induced by H2O2. When the cells were allowed to enter plateau phase growth (to mimic differentiation), no differences in repair rates were observed between actively growing cells and plateau phase cells, whereas plateau phase cells had reduced levels of SSBs.

It has been noted that high LET radiations are more effective at inducing cataract compared to low LET X-rays, potentially because of the increased complexity of DNA damage [116]. It should also be noted that the differentiated LFCs, in addition to LECs, are also subject to oxidation that can lead to modification, denaturation and aggregation [59].

*3.2. Gene expression changes in response to IR*

 There is evidence that DSB repair deficient ataxia telangiectasia mutated (*Atm*+/– and *Atm*–/–) mice demonstrate earlier, increased incidence of cataract post IR when compared to the wild type [19,117]. Oxidative stress as an insult alone has also been shown to alter gene expression in LECs deficient in peroxiredoxin-6 (PRDX6), taken from mice which went on to develop cataract [118]. This protein functions to maintain cellular integrity and regulate cell differentiation and proliferation through the stabilization of ROS levels. When this regulation was lost, physiological signaling was attenuated, resulting in a loss of homeostasis and modified gene expression. Altered gene expression included TGFβ, which in turn drove the expression of tropomyosin (Tm) 1 and 2 (cytoskeletal proteins) in LECs [109,118]. It has been suggested that, if it could be observed *in vivo*, this could be a useful clinical marker for PSCs, although non-invasive biomarker assessment is a complex task. Xeroderma pigmentosum D (XPD) that functions in the NER pathway has also shown some polymorphisms associated with age-related cataract, although in some cases, the risk was actually decreased [119].

 Hamada and Fujimichi have recently reviewed some of the mechanisms behind IR induced cataract: they discussed several studies that had investigated a number of genotypes that either showed no or reduced expression of various carcinogenesis-linked proteins, or in some cases genotypes that were nullizygous/heterozygous [76]. Genetic studies identified polymorphisms in the genes coding for the X-ray repair complementing defective repair in Chinese Hamster cells 1 (XRCC1) and 8-oxoguanine DNA glycosylase 1 (OGG1), which function in the BER pathway that could be associated with age-related cataracts [76].

 When gene expression was analyzed *ex vivo* in human age-related cataracts compared to transparent lenses, the genes that showed differences were chiefly downregulated. Only 6 genes were upregulated in humans, none of which matched those described above [120]. Single nucleotide polymorphisms (SNPs) in some of the genes associated with cataract may increase or decrease sensitivity to development of age-related cataracts [121]. This suggests that one or more different mechanisms may account for IR induced and age-related cataract, potentially explaining the varying prevalence of the different types of cataract. A number of cataractogenic mutations have also been identified in human crystallin genes [93,122,123].

 As discussed in section 2, LFC formation is a highly orchestrated process, with large numbers of proteins involved [124,125]. Alterations to this process have been noted after heavy ion IR exposure, including to the cyclin-dependant kinase inhibitor 1A (*CDKN1A*): irradiation with 4 Gy of iron ions of different LETs upregulated *CDKN1A*, apparently as a linear function of LET [60]. Human lenses were examined post mortem for the localization of *CDKN1A* which was observed in the TZ, suggesting a role in the differentiation from LEC to LFC. *CDKN1A* has also been implicated in cell adhesion and therefore interaction with ECM [60]. A number of other cell cycle associated genes were found to be upregulated and a few were downregulated. It was concluded that this altered gene expression pattern was responsible for premature elongation and alignment as well as the premature expression of adhesion markers. FGFs have long been known to be important for LFC differentiation [126]. For instance, FGF2 was increased post 4 Gy irradiation in a cyclical trend. Interestingly, human embryonic stem (hES) cells could be stimulated to form lentoid bodies in a system using FGF2 to direct differentiation [127].

 The role of MMPs has also been investigated in cataract [128] and following IR [60]. These proteins are capable of degrading a wide range of ECM proteins, which have also been implicated in cellular processes such as apoptosis, cell adhesion/dispersion and differentiation, and activity increases with age in LECs from patients with age-related cataracts [128]. In a study investigating both bovine LECs and a human cell line, LECs were shown to derive matrikines (ECM bound growth factors) from the lens capsule [62]. A gradient of available FGF2 [61] determined the compartmentalization of the epithelium according to this gradient [32]. Following irradiation, LECs showed a downregulation for certain MMPs. Ultimately, IR exposure and its downstream effects (e.g. cytokine signaling) and growth factors (e.g. FGF2) can be held responsible for these ECM-related changes in frog models (e.g. [129]), and such events are thus likely to be important to understanding the mechanisms of IR action.

*3.3. Morphology*

 *In vitro* culture of lens progenitor cells (generated from hES cells) leads to the formation of lentoid bodies [127]. When human LECs were grown in plastic, after an extended period of time, primary cells began to elongate and align in a parallel fashion, consistent with differentiation into LFCs *in vivo* [108]. Evidence for enucleation in intact cells has also been observed after 15 days in culture [108]. There have been a number of studies on LEC morphology *in vitro* following UV exposure. Imaging of a UV exposed human LEC cell line revealed a number of processes including apoptosis, nuclear membrane shrinkage, chromatin condensation, fragmentation, but also necrosis, swelling of the nucleus and cell body and disruption of the plasma membrane. It also revealed phagocytosis and migration of neighboring cells. This suggests that LECs respond to signals in the microenvironment, promoting cell movement [130].

#  Although the lens is a closed system, and apoptosis is thought to be a rare event [32], it has been suggested that LFC organelle loss has many parallels with classical apoptosis [31]. MicroRNA-125b has been shown to inhibit LEC apoptosis by targeting p53 in age-related cataract [131]. Xie et al. (2014) also noted other epigenetic mechanisms implicating histone deacetylase in the prevention of EMT through TGFβ modulation [132]. IR can also cause severe decreases in the number of surface LECs, altering proliferation and differentiation rates [107] and effectiveness [97]. In general, it is hypothesized that X-irradiation can induce age-related abnormalities at an accelerated rate [18]. A similar hypothesis is used to explain UV accelerated aging of the skin [133].

 SRA01/04 cells (human LECs immortalized with simian virus 40 T antigen) were subjected to 2 week exposure to 50 µM H2O2with the aim of simulating oxidative stress [102]. As well as a number of biochemical alterations, the cells demonstrated gross enlargement, flattening, and the accumulation of granular cytoplasmic inclusions typical of the senescence process common to many tissues [134]. Changes were visible as early as 3 days after starting the treatment. When N-myc downstream regulated gene 2 (*Ndrg2*) was overexpressed through adenoviral infection, as it was by H2O2 treatment, the cells started to show altered morphology with characteristics including cellular stretching, followed by a fibroblast-like appearance 12 h post infection with the *Ndrg2* adenoviral vector, by 48 h the majority of cells displayed a fibroblast-like appearance [132]. However, the Mouse Genome Informatics database does not reveal an obvious link between *Ndrg2* and the eye [135].

 A potential candidate mechanism for the lens senescence leading to cataractogenesis is telomere shortening/lack of telomerase. It has been hypothesized that the rate of telomere shortening associated with aging and cataract formation depends on both oxidative stress and inherent antioxidant capabilities – leading to oxidative and lipid peroxidative damage [136]. Telomere length in leukocytes has even been suggested as a marker of aging (by association with lens transparency), though no association with clinical cataract outcome was identified [89]. Further, recent reviews of the association between oxidative stress and cataract have also indicated a role of premature senescence [54]. It has been suggested that such biomarkers of oxidative stress can be considered as general biomarkers for life expectancy in veterinary circles [137], and cataract treatments focusing on prevention of loss of functional telomere length (also discussed in section 5) are already in development [54]. Telomeres and senescence have been implicated in accelerated aging [138] which is also a suggested mechanism of cataractogenesis [139].

*3.4. Lens fiber protein changes*

 In addition to altered cellular morphology, changes in the crystallins that make up the majority of protein content within the lens have been noted following IR exposure [105]. In a recent study of DNA damage following X-ray exposure [107], surviving LECs in mice showed excessive proliferation, potentially an effort to repopulate those cells that were lost. Cell differentiation and proliferation are a precisely regulated mechanism for effective lens development and maintenance; any disruption to this process may lead to problems and potential opacities [31,99,100]. Peripheral proliferating LECs are thought to be radiosensitive [107] and have less effective means of repair. Impaired DNA damage repair also enhances the progression of spontaneous cataractogenesis regardless of IR exposure [76]. Although it is possible that this may be a result of the immortalization process, if similar occurrences are observed in lenses *in vitro*, such excessive growth could have implications *in vivo* for cataractogenesis. Indeed, Costello et al. observed increased numbers of multivesicular bodies in age-related cataract [140] which may be a distinguishing phenotype of this type of cataract.

 Protein aggregation and the effect upon the lens transparency/scattering of light are one of the final steps in formation of lens opacities [141], and the issue is reviewed in detail by Hickman et al. [123]. Lens fiber proteins usually exist in highly ordered and structured patterns which help with the maintenance of lens transparency [142]. A large body of *in vitro* work has demonstrated the contribution of protein folding pathways to cataract formation (e.g. [143]). Further, the lens has relatively large amounts of aromatic and sulfur amino acids which are known to make protein structures more vulnerable to radical damage [144]. γ-rays have been linked to alterations of the structure of crystallins through oxidative stress and alterations in the surface hydrophobicity, leading to the accumulation of crystallin aggregates [105]. Deamidation has been suggested to contribute to protein unfolding and aggregation in lenses by decreasing stability of human crystallins [17,145,146], and the same can be said for other proteins expressed in the lens [147]. α-crystallins also have a chaperone function preventing the aggregation of other proteins; however, γ-irradiation abrogated this function likely through the oxidative damage to tryptophan residues, particularly kynurenine [105,148]. Lens intrinsic membrane protein 2 (Lim2/MP20), an important LFC membrane protein, has also been implicated in maintenance of cytoskeletal integrity, cell morphology, and intercellular communication [149]. Further, it has been shown that during lens cell differentiation, alterations in the organisation of the lens fiber cell cytoskeleton occur, in particular the lens-specific intermediate filament network upon which cell shape, polarization and general tissue integrity all depend [150,151].

*3.5. Non-targeted effects*

 Here we define non-targeted effects (NTEs) as any effects occurring in targets (e.g. cells) that have not been directly irradiated. Although abscopal effects (out of field effects; biological changes seen at distant sites from a point of irradiation but occurring as a result of that exposure through some form of signaling) were first discussed in the context of radiotherapy in the early 1950s [152], localized NTEs are a relatively new but increasingly well-defined concept in radiobiology, which stand alongside and in parallel to the traditional target theory. NTEs are generally classified in two different ways: IR induced genomic instability (RIGI) occurs in a temporal manner, i.e. in the descendants of irradiated cells that once appeared free from damage post irradiation. In addition, IR induced bystander effects (RIBEs) occur in a spatial manner, i.e. in cells that received no IR exposure but have communicated with cells that received IR exposure [153].

*3.5.1. Ionising radiation induced genomic instability*

 RIGI occurs downstream of the initial IR insult, in the progeny of the irradiated population. Oxidative stress is a critical early factor in RIGI that can initiate the instability phenotype. IR can cause a low level of oxidative stress that results in new point mutations (insertion, deletion or substitutions) and DNA strand breaks [154]; mutations in critical genes that could lead to a mutator phenotype [154]. Oxidative damage associated with reactive oxygen species (ROS) produced from lipid peroxidation [155] and by phagocytes [156] is one of the main causes of spontaneous DNA damage at the basal level. IR can also induce oxidative DNA base damage, leading to damage in hemopoietic cells due to vulnerability to free radicals [157]. Increased ROS has also been linked to delayed death of Chinese hamster ovary (CHO) cells as a result of IR exposure leading to necrosis and apoptosis [158]. This is one example highlighting the relationship between IR induced oxidative stress and RIGI.

 It is assumed that LECs in the GZ are the most sensitive to IR exposure (e.g. [107]), and therefore these cells would be most likely to develop an instability phenotype. The fact that these cells can divide potentially allows them to transmit the unstable phenotype, possibly deregulating the tightly controlled lens differentiation process [159–162]. The manifestations of RIGI also correlate with the effects seen in cataract [163] such as DNA damage, oxidative stress and altered gene expression through epigenetic mechanisms [164].

*3.5.2. Ionising radiation induced bystander effect*

 The link between hit and non-hit cells after IR exposure *in vivo* and *in vitro* has been observed in different cell types after high- and low LET exposure [153]. Although the mechanism is not fully clear yet, it is reported that the communication between irradiated cells and unirradiated cells could be through direct intercellular communication via GJs stimulating a damage-signaling pathway induced by p53 [165], or through release of soluble signaling, such as ROS and reactive nitrogen species (RNS) [153] (although they generally have relatively short lifetimes [166]), cytokines [interleukin (IL)-8, tumor necrosis factor α (TNFα) and TGFβ], and finally extracellular vesicles like exosomes [167]. The fact that IR exposure directly affects GJ-mediated intercellular communication (of free radicals, intracellular calcium and proinflammatory cytokines; reviewed in [168,169]) indicates that these mechanisms may play an important role in NTEs. Intracellular Ca2+ signaling, for example, has been shown to be slower in cells taken from surgically isolated lens capsules associated with the most highly developed cataracts [90,170] – indicating a role for altered Ca2+ signaling in cataractogenesis.

 There are few data, particularly *in vitro,* examining RIBEs in LECs. However, it could be easily speculated that these effects may be involved in the process of cataractogenesis. Hickman et al. [171] were the first to report that p53 mediates RIBEs after exposure of rat lung epithelial cells to low dose α particles. Moreover, a study has reported that following IR, irradiated cells released cytokines and prostaglandin E2 via autocrine/paracrine mechanisms, which in turn activated the expression of IL-6, TNFα, IL-8, cyclooxygenase 2 (COX-2) and IL-33 which are essential for signaling pathways [172]. Following IR, free radical production could stimulate cytokine production in the lens as observed in prostate tumor cells: for example, TNFα that activates IκB kinase (IKK)-mediated phosphorylation of inhibitor of nuclear factor (NF) κB (IκB), which in turn secretes NF-κB that enters the nucleus and acts as a transcription factor for COX-2 and inducible nitric oxide genes [173].

 LECs can signal to themselves and neighboring cells through autocrine, paracrine [174,175] and to neighboring cells through GJs [126,176]. RIBEs can also occur through GJs [177]. In early lens development, Cx50 is the most common Cx GJ channel, with other connexins (Cx43, Cx46) also being involved in growth and maintenance of lens transparency [176,178,179]. These protein Cx channels have been heavily implicated in RIBEs observed in other cell lines [59,180].

**4. Recent evidence from *in vivo* animal models**

*4.1. How to measure cataracts?*

 Investigations into cataract formation following IR exposure have been performed since the end of the 19th century. For decades, the method of choice was the eye examination by a slit lamp which allowed a stereoscopic magnified view, particularly of the cornea and the lens. Researchers initially used a system of four grades of cataracts [181] and, in 1962, Merriam and Focht introduced (in rats) a grading system to standardize description and classification of cataract formation from an increase in the light reflex at the posterior pole of the lens (stage 1+) to a completely opaque lens (stage 4+) [8]. Intermediate stages were defined as stage 2+ by a moderately dense posterior cortical opacity with some early opacifications of the anterior cortex, and stage 3+ by a very opaque posterior cortex and a moderately opaque anterior cortex. At this stage, the lens nucleus often showed early sclerosis. However, this system was not universally accepted: for instance, some researchers still used a three-grade system [182]. Moreover, it is obvious that investigation by a slit lamp is observer dependent and thus subjective (albeit with the potential for observers becoming highly skilled); nevertheless, due to lack of alternative methods, this method has been used for mice, rat and human studies for several decades. The objective monitoring of cataract formation (in terms of the percentage of lens opacity) was first reported in 1984 by Hockwin et al. [183] with the introduction of the Scheimpflug system. This new method was used in rats [183] and later in mice [184]. For high-throughput screening in mice, the first systematic application of a Scheimpflug camera was demonstrated by Puk et al. [185]. A new method based on dynamic scattering of light has also been recently developed [186], and used in human studies of age-cataract development [187] for example, but not yet for IR induced cataract. Ocular coherence tomography [188], perhaps used in addition to dynamic light scattering [189], may also hold potential for future cataract detection/classification.

 For comparison with human eye development, it is important to know differences between human and murine lenses [190]. However, at birth, mice reach a similar developmental stage as a human embryo at the beginning of the third month of age, i.e. degradation of organelles in the LFCs. The time when the mouse opens its eyes (i.e. 2 weeks after birth) correlates with the beginning of the third trimester of human embryos [191–193].

*4.2. Dose dependence of cataracts*

 To date, most experiments on IR induced cataracts have been performed in mice and rats at doses ≥1 Gy. One of the most comprehensive studies was published by Upton et al. in 1956 [194] comparing the effectiveness of neutrons, X-rays and γ-rays for the production of cataracts in mice (RF strain), rats (Wistar), guinea pigs and rabbits after whole body irradiation. They observed decreasing radiosensitivity in the lens in the above order of species; dose fractionation had only a slight if any influence in cataract formation. They also showed that dot-like vacuoles in the posterior subcapsular region along the suture (grade +) are formed in the irradiated mice earlier (~8 months) than in the controls (~13 months) even at 33 rep (“Röntgen equivalent physical” unit, equivalent to approximately 0.3 Gy; dose rate 3 R/min) for X- or γ-rays; however, 2 years later, cataract formation did not advance beyond those developing in the controls at the end of the study (when most cataracts had reached grade 2, with larger vacuoles interspersed among radial streaks). The highest dose used in this experiment was 333 rep (~3 Gy) leading to cataracts of grade 3 (anteriorly extended streaks with expanding anterior subcapsular opacities) 2 years after irradiation. Neutron irradiation was approximately 9 times more efficient than X- or γ-irradiation. With X- or γ-rays, they did not observe mature cataracts (grade 4) in mice [highest dose 640 rep (6 Gy) by X-rays or 800 rep (7.4 Gy) by γ-rays]; however, 640 rep (6 Gy) applied to rats produced mature cataracts [194]. In the same year, Riley et al. [195] reported cataract formation of grade 1 (posterior polar vacuoles) 1 year after irradiation of the heads of albino Swiss mice with 400 R (3.7 Gy) of X-rays (dose rate 33 R/min; 200 kVp); stage 2 (partially diffuse opacity) was observed at ~700 R (6.5 Gy) and stage 3 (complete opacification) at 1200 R (11 Gy).

 In 1975, Schenken et al. reported a very detailed experiment with 14 different X-ray exposure regimes using several different doses applied as a single dose or in fractions to the head of male HA/ICR mice at the age of 14-16 weeks (dose rate 130 R/min; 1.1 Gy/min) [196]. Six months after a single dose of 900 R (8.4 Gy), they observed cataracts of grade III (complete cataract) in 80% of the mice, and in 100% of the mice after 1100 R (10.2 Gy). Dose fractionation led to higher total doses necessary for complete cataract formation, indicating some repair processes in the lens (LECs). Unfortunately, they did not give data of doses leading to grade I or grade II cataracts.

 It is also noteworthy that no IR induced lens changes were found in goats after γ-irradiation (4.0–4.7 Gy), even 3 years after irradiation [182].

 In summary, the high variation in the genetic background of the mice and other animals used in the different experiments as well as differences in the IR schemes and grading systems make it very difficult to compare the results of different studies.

*4.3. Genetic effects and strain dependence of radiation induced cataracts*

 Since a broad variety of hereditary cataracts are well established in humans and mice (reviewed in [93]), it might be expected that environmental influences including IR might have cooperative effects in cataract formation with the underlying genetic background. Examples are reported by Worgul et al. [19,117] and Kleiman et al. [197], who irradiated 4-week old *Atm*+/– or *Atm*–/– mice. At the lowest dose (0.5 Gy, dose rate 0.5 Gy/min), 50% of the wild-type mice developed grade 1 cataract (according to [8]) 30 weeks after irradiation, compared to 90% of the heterozygotes [197]. Similarly, at 1 Gy and 2 Gy, 50% of the heterozygotes developed cataracts (grade 1) earlier than wild types; however, at 8 Gy, there was no difference between wild types and heterozygotes. This indicates that the genetic difference becomes more important as dose decreases [19]. The genetic effect increased when *Atm*+/–/*Mrad9*+/– mice were irradiated with 0.5 Gy; they developed cataracts of grade 1 and of grade 2 much earlier than wild types. Mice heterozygous at one of the two genes are intermediate in sensitivity. However, there had been no systematic evaluation of strain dependent sensitivity or resistance to IR induced cataracts.

 More recently, De Stefano et al. [198] analyzed the formation of total cataracts in heterozygous Patched 1 (*Ptch1)* mutants (on CD1 background) after irradiation with 3 Gy at 2, 10 or 56 days after birth. They defined cataracts as a white pinpoint focus grossly visible in unpigmented eyes. Over the entire lifetime, they did not observe such total cataracts in unirradiated wild-type mice, but in 6% of unirradiated heterozygotes with a median latency of 34 weeks. When irradiated at an age of 10 or 56 days, no bilateral cataracts were observed, either in wild-type or in heterozygous mice. Only in lenses irradiated at 2 days of age, bilateral total cataracts have been observed (14% in heterozygotes and 2% in wild types); the median latency to cataract formation was 6.9 weeks in heterozygotes and 9.5 weeks in wild-types. It should be noted that, similar to black Swiss mice, CD1 mice are also outbred mice resulting in a higher genetic heterogeneity than inbred strains of mice ([199]; http://www.criver.com/).

 These experiments indicate that heterozygosity for *Atm*, *Mrad9* or *Ptch1* increases sensitivity to IR induced cataracts. However, the differences in the genetic background of the controls and the mutant lines, together with the differences in the age at irradiation and the different endpoints in cataract formation, make comparison between the studies difficult.

*4.4. Age of animals at the time of irradiation*

 For mice, one study was published concerning the age dependence of cataract formation using a single dose of 300 R (2.8 Gy; dose rate: 40 R/min) in inbred mice. The most radiosensitive period was the first 3 days after birth. Within the next 2 weeks, the lens became more radioresistant in terms of lens opacification, and after 3 weeks of age, it took 34-45 weeks until lenticular lesions occurred in both eyes. At the age of 6 months, the latency period was 56 weeks, and mice irradiated at approximately 1 year of age developed cataracts at the same age as the unirradiated control (i.e. at age 85 weeks [200]).

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Table 2. Cataract appearance after exposure to ionizing radiation (X-ray and γ-irradiation).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| First signs of cataract (dose; time after irradiation) | Applied dose | Cataractogenic threshold dose | Observation period | Cataract | Energy | Dose rate | Age of exposure | Mouse strain | References |
| 0.3 Gy (33 rep);8 months | 0.3-3 Gy (33-333 rep) | 4.65-2.79 mGy(0.15-0.30 R) | 36 months | Grade 1-4 | 250 kV | 0.65-0.74 Gy/min(70-80 R/min) | 8-14 weeks;6-12 weeks | RF;LAF1 | Upton et al. 1956 [8] |
| 0.93 Gy (100 R);1 year | 0.93-9.3 Gy(100-1000 R) | Not calculated [CD50: 7.21 Gy (775 R)] | 1 year | Grade 1-3 | 200/ 250 kV | 0.31 Gy/min(33 R/min) | 10-14 weeks | Swiss albino | Riley et al. 1956 [195] |
| 0.5 Gy (50 rad);117 days | 0.5-4.56 Gy(50-456 rad) | Not calculated | 500 days | 2%, 10% lens density | 300 kV | 0.56-0.84 Gy/min(60-90 R/min) | 8 weeks | RF | Darden et al. 1970 [201] |
| 8.37 Gy (900 R); 6 months (grade 3 cataracts) | 6.51-20.46 Gy(700-2200 R) | Not calculated [CD50: 7.77 Gy (835 R)] | 6 months | Grade 1-3 | 270 kV | 1.12 Gy/min(130 R/min) | 14-16 weeks | Ha/ICR | Schenken, Hegemann 1975 [196] |
| Complex age related | 2.79 Gy(300 R) | Not calculated | Throughout life (>700 days) | Grade 1-5 | 170 kV | 0.39 Gy/min(42 R/min) | 1-7 days1-52 weeks | A | Gajewski et al. 1977 [200] |
| 0.5 Gy; 10 weeks | 0.5-4.0 Gy | Not calculated | Throughout life | Grade 1-4 | 250 kV | 0.5 Gy/min | 4 weeks | *Atm+/-; Atm-/-*129SvEv, Black Swiss | Worgul et al. 2002 [117] |
| 0.5 Gy; 10 weeks | 0.5 Gy | Not calculated | Throughout life | Grade 1-4 | 250 kV | 0.5 Gy/min | 4 weeks | *Atm+/-; Mrad9+/-*129SvEv, Black Swiss | Kleiman et al. 2007 [197] |

To aid comparison, historical units were converted to gray (Gy), information from the cited papers were put in parentheses. 1 rad = 10 mGy. 1 rep, (röntgen equivalent physical) ≈ 9.3 mGy. 1 R (Röntgen) = 2.58 x 10-4 C/kg ≈ 9.3 mGy. Different grading systems for cataracts were used (see section 4.1).

**5. Evidence from mechanistic studies in low dose exposed and other relevant populations**

 Although the recent advances in epidemiological studies will be reported in a separate publication in this issue [21], epidemiological incidence data can certainly contribute to knowledge and understanding of IR effects, for instance, the observation that PSCs are the most common form of IR induced cataract ([139]). Indeed, such data form the basis of radiation protection. There have also been a number of epidemiological studies that have combined some mechanistic information. For instance, following the discussion of the potential role of telomeres in cataractogenesis in section 3.3, shorter telomere lengths in peripheral blood lymphocytes were observed in Chernobyl accident clean-up workers diagnosed with cataract [202] 23 years after recorded doses up to 300 mSv; however, there was no significant association between telomere length and dose [203].

 Following ICRP recommendations [12], there has been a particular interest in medical workers exposed to IR, including interventional radiologists and cardiologists, as these individuals can certainly receive lens doses in the region of 20 mSv per year or indeed rather more than this in some circumstances [204]. A 20-year study based on almost 36,000 radiologic technologists in the US was published in 2008 [139]. An increased incidence of cataracts was found in workers, of whom the majority received a cumulative dose lower than 500 mGy. However, an important point here is that the dosimetry and exposure data were assessed by questionnaire, which leads to large uncertainties. The accuracy of dosimetry, especially at low doses, is known to be a common problem in epidemiological studies [3]. Findings from a recent review of lens exposures and effects in medical workers suggest that doses on the order of a few mSv may indeed lead to measurable cataracts [205]. For instance, a recent study of 81 interventional cardiology staff in Iran showed a relative risk of opacity compared to non-IR exposed nurses of approximately 11 [206]. This clearly supports the judgement of ICRP that the threshold for cataract is lower than previously thought and may further suggest a lower threshold or stochastic mechanism for cataract development [12]. Further, in 2013, Jacob et al. [98] found significant increases in PSCs in (albeit small numbers of) interventional cardiologists compared to age-matched unexposed controls, again echoing previous findings in lower dose exposure settings. It might be interesting to note here that most human studies are carried out in the absence of genotyping thus, given that *Atm* heterozygotes, for example, account for a few percent of the whole population, it is possible that genetic background may have a confounding effect [207].

 Further specific mechanistic details are sparse in human studies; however, there is direct evidence in individuals or human populations. As discussed in more detail in section 3, it is strongly suspected that oxidative damage plays a role in cataract initiation or progression. Indeed, DNA methylation and gene expression upstream of DNA repair have been implicated: OGG1 levels were recently found to be significantly reduced in age-related cataract patients [208], although the evidence for a role of BER or NER genes in age-related cataract is conflicting [76] and there is no human evidence for IR induced cataracts to date. Genetic polymorphisms in DNA repair genes are also associated with an increased risk of age-related cataract in Chinese populations [209] and limited data suggest that *Atm* haplotypes were associated with an increased risk of IR cataractogenesis in atomic bomb (A-bomb) survivors [28]. Insufficient repair of DNA damage may play a role, but the human evidence is thus far limited to a very small number of studies, for example, demonstrating lens opacity in Nijmegen breakage syndrome (NBS) patients, who have inherited mutations in the nibrin (*NBN*)/*NBS1* gene [210] which is critical in DNA damage responses [211].

 Tumor related factors potentially associated with cataractogenesis have recently been reviewed [76]. Human studies have demonstrated a potential role of mutations in oncogenes, tumor suppressor genes and other tumor related genes. Examples include the *MAF* gene, an oncogene which regulates expression of crystallins and has a key role in embryonic LFC differentiation, in the development of congenital eye disease including cataracts (e.g. [212]); polymorphisms or mutations in genes encoding the ephrin receptor tyrosine kinase type A2 (*EPHA2*), e.g. [213]; various polymorphisms in genes of glutathione S-transferases increase the risk for age-related cortical cataracts particularly in smokers, for example [214].

 Delving deeper into potential mechanisms, negative studies also have the potential to be informative. For instance, glycation and lens autofluorescence due to glycosylation proteins leading to aggregation and unfolding of lens crystallins have long been associated with diabetes mellitus and nuclear cataract [215,216]; however, there is no evidence that autofluorescence is associated with posterior subcapsular opacities [217]. Lens autofluorescence is now proposed to be a bioindicator for diabetes type 2 [218]. Nutrition has also been shown to play a role in PSCs, though it is likely a more important factor for nuclear cataracts [219], and steroid treatment certainly leads to increased numbers of PSCs [220].

 Intercellular communication has been suggested to play a role in progression of cataractogenesis, as discussed in section 3. However, direct human studies would be extremely difficult to perform, so there is very little evidence from individual human or population studies. There is, however, limited evidence for the role of the cellular immune response in general cataractogenesis: for instance, lens protein antibodies are frequently found in lenses extracted from patients with age-related or diabetic cataracts [221]. In addition, inflammation has been shown to play a role in the A-bomb induced cataracts [222]. Furthermore, ocular inflammation treated with steroids results in a high risk of cataracts (particularly PSCs or posterior subcapsular opacification) [220]. This is also of interest in the context of the discussion on NTEs in section 3.

 Although LECs in culture demonstrate very low rates of apoptosis [32], apoptosis has been associated with general development of non-congenital cataract in human populations [223], though this remains to be demonstrated following IR exposure. In general, it is thought that apoptosis is not likely to play a major role in IR cataractogenesis, although autophagy has been proposed to play a role in lens organelle degradation [224]. However, cell death can still occur through inactivation and cell cycle arrest in some circumstances. Accumulation of small scale LEC losses in human lenses in age-related cortical cataract may induce alterations leading to reduced transparency [225].

 Finally, associations between other risk factors and confounders in epidemiological studies have been reported in a number of different populations – for instance, PSCs are known to be less common in aging than nuclear cataracts, however in some populations, PSCs are more common in younger age groups [226]. This issue is discussed in detail in section 7.

<Figure 2 here>

**6. Dosimetry aspects**

*6.1. Dose and dose rate*

 To understand the risks associated with IR exposures, it is necessary to identify the effect of the IR and also to quantify the IR exposure that caused these effects. This became apparent in the early days of experimentation with and application of IR, resulting in establishment of two bodies, ICRP and the International Commission on Radiation Measurements and Units (ICRU), which define quantities associated with IR exposures. The importance of the eyes in radiation protection was recognized early in ICRP recommendations which considered “doses to the gonads, the blood forming organs and the lenses of the eyes” as being of primary importance [6,227], with a recommended depth for the dose being specified as 3 mm, though that early recommendation was not accompanied by any specific advice on how to limit dose to the eye lens.

 However, a key issue common to many epidemiological studies, in particular, is that high quality dosimetry at low doses as part of epidemiological studies is very difficult to obtain, with frequent use of questionnaires relying on recall, or generic calculations [11,228,229]. Although direct methods of dosimetry are now available (e.g. by Gilvin et al. [230]), the latency period, especially at low doses, means that epidemiological studies of cataract development take many years, and thus it is likely that it will be some time before large scale studies with truly reliable low dose dosimetry is available. Further, although it is possible to achieve high levels of accuracy in dosimetry in cellular and animal studies, the relevance of doses applied in requires further consideration.

 As with most tissues and biological endpoints, the effectiveness of a dose of low LET IR for induction of cataracts is reduced if the dose is fractionated or protracted over time; however, it should be noted that data regarding lens effects have been gathered from studies not designed to address this. Evidence for the sparing effect of fractionation or protraction comes not only from animal experiments, but also from clinical experience. For example, a significant reduction in cataract incidence is observed in patients that have received total body irradiation for bone marrow or stem cell transplantation if the dose rate is decreased [231,232].

 The sparing effect from fractionation is only observed after exposure to low LET IR, which is the key focus of this review. However, more densely IRs show little to no dose rate effect [233]. The lack of a dose rate effect for the lens after exposure to high LET radiations is likely due to the differences in the DNA damage species produced, compared to low LET radiations, as well as less proclivity for repair after exposure. The companion paper in this issue further reviews the impact of high LET IR on the lens [20].

 Interestingly, however, for populations receiving low, protracted occupational exposures over long periods [139,234] or exposed to natural high background levels [235], no reduction in incidence is noted when compared with populations exposed to single dose exposures. Since chronic low dose rate exposures could result in minor changes, particularly in lenses of younger individuals [235], one cannot assume that there would be a decreased cataract hazard risk after exposure to low doses of IR over months, years or over an individual’s entire lifetime.

 ICRP’s current position is that there is no dose rate effect for cataractogenesis but this is because of a lack of evidence [12]. However, there is evidence that protracted exposures do lead to significant elevation of cataract incidence [235]. Thus, this issue clearly requires further consideration.

*6.2. Modeling energy deposition in the lens*

 Understanding the nature of the energy deposition by IR in the lens requires computer models, because it is not possible to measure it *in vivo*. Ideally, it would be possible to use analytical methods to determine the energy deposition or ionization in the lens, cornea and aqueous humour (see below) but the problem is too complex to solve for any realistic geometry. Instead, it is necessary to use the Monte Carlo method [236] where random histories are used to sample the problem, until statistical convergence is reached. The method is robust, but requires good physics models, accurate interaction data for IR with matter and powerful computers. These have all been developing over the last 70 years, but it is really only in recent times that accurate models of the human eye have been feasible.

 Recognition that the eye needed accurate description goes back to at least ICRP Publication 23 [237]: though prior models of the human body used for IR transport existed, they did not have defined eyes [238]. These computational phantoms used an assembly of geometric shapes to describe the human body, but the range of shapes available was limited, and the addition of more details would slow the computation significantly: descriptions of small features such as the lens were not accurate. For radiation protection, specific “home-made” Monte Carlo codes were generally used with crude anthropomorphic phantoms [239,240], though increasingly more general purpose codes were becoming available to do the same job.

 Despite the earlier recognition that eye lens doses were of concern for radiation protection, definitions of dose quantities that would enable dose deposition to be calculated in the eye or eye lens took a long time to catch up. ICRU considered only superficial (skin) and penetrating (whole body) dose equivalent [241], and ICRP used a phantom with no eyes [242], though conversion coefficients for 3 mm depth were plotted but not tabulated. The Medical Internal Radiation Dose (MIRD)-5 phantom, which had no eyes, was adapted for radiation protection [239,243]. This enabled the calculation of lens doses from external photon sources [244], though the eyes in the models were little more than slabs of tissue attached to the head of the phantom. No detailed description of the lens was attempted.

 Computations of lens doses in anthropomorphic phantoms for external photon exposures were used to obtain reference conversion coefficients in a joint ICRP/ICRU report on protection quantities [245]. This used data from several sources: computations with own codes (e.g. [246,247]) or using the generic Monte Carlo N-particle (MCNP) code for transport of radiation through various materials [248], but none of the models used included realistic descriptions of the human eye: at that time, although the importance of the lens as a tissue affected by the body was recognized, it was also of relatively low significance in radiation protection because its dose limit being 150 mSv compared to 20 mSv for whole body effective dose, meant that it was rarely limiting in terms of occupational exposures. Further, the computational hardware, codes and methods available were not capable of accurate calculations on small scales. MCNP version 4 [249] could transport neutrons and photons, but not electrons, which made it suitable for many large scale problems, but on a small scale dose is deposited by secondary electrons, so computation of doses on the scale of the lens was inherently inaccurate. This omission would not matter where materials were uniform in composition, but in the lens, transport of secondary electrons from photon exposures is necessary for accurate dosimetry.

 In the 1990s, the “EGS4” code [250] was showing much improved electron transport [251], but this still used group approximations to address the problem that it was not possible to follow individual electron histories. This remains a problem for most codes that operate on macroscopic problems, though now the electron transport available in “MCNP6” [252], “EGS-NRC” [253], “PHITS” [254] and “PENELOPE” [255] is much enhanced and able to cope with significantly smaller structures: if the scale on which the dose needs to be calculated is small, average properties of groups of electrons are not sufficient, especially where there are boundaries between different materials.

 The older computational phantoms constructed from geometric shapes have been replaced in radiation protection by voxel (smallest computational element) phantoms based on computed tomography (CT) scan images [242]. There are reference male and female computational phantoms with voxel sizes of 2.137 x 2.137 x 8.00 mm3 and 1.775 x 1.775 x 4.84 mm3, respectively, which are far too coarse for accurate representations of some features such as the lens. For this reason, a better model of the eye was developed based on published data [256] that simulated the lens with better accuracy than any prior model [257,258]. The model includes descriptions of the lids, cornea, anterior chamber, vitreous body, insensitive part and the sensitive part of the lens. Different material compositions and densities were used for the lids, lens, aqueous humor, vitreous humor and the cornea [259]. The distance of the surface of the “sensitive” volume of the lens varied from 2.71 mm to 3.70 mm, with the average being 3.25 mm, slightly deeper than the 3 mm used for radiation protection.

 It was acknowledged that there was insufficient information about the appropriate thickness for the sensitive part of the lens for a realistic description, so the characteristics used to define the sensitive region was varied to assess the sensitivity of the dose deposition on the thickness of the presumed sensitive layer. Because cones were used to define its shape, the thickness of the sensitive volume varied from zero at its center to about 0.45 mm near its edge. Increasing the thickness by factors of 1.25 and 1.50, produced reductions in the peak dose deposition by electrons of 8% and 12%, respectively [258], but because thinner sensitive volumes were not used, it is not possible to infer accurately what effect would have been seen. Subsequent calculations comparing the dose deposited in the whole lens and the sensitive volume [260] showed no difference for photons, which is a surprising result, given the previously published difference for electrons for small changes in the sensitive volume. The paper makes no reference for how the secondary electrons were transported and was missing important details regarding how doses were defined (in particular, whether the kerma approximation was used). The electron data in the paper show significant differences, which is to be expected given the difference in the materials of the eye, and the change in the stopping power of electrons on these small scales.

 ICRP’s reference conversion coefficients for external IR [261] use this detailed model of the eye embedded in an average of the Adam and Eva mathematical phantoms [239] for photons and electrons and an Oak Ridge National Laboratory (ORNL) mathematical phantom [262] for neutrons [263]. The data for photons now show a 33% difference for 10 MeV photons between the whole lens and the sensitive volume, that demonstrates the critical importance of getting the secondary electron transport right. The difference would be larger if a thinner sensitive layer was used. The difference for electrons is a factor of 5 for some energies, whilst no significant difference is observed for neutrons. Data are also given for the reference voxel phantoms that should perform inadequately because the lens is poorly described. For photons, little difference is observed at lower energies, though for high energies issues of secondary charged particle equilibrium cause the voxel phantom data to be much too high. However, for incident electrons at low energies, there are big differences that demonstrate the importance of accurate models. For neutrons, there is no discernible difference between the data for the reference voxel phantom models, the whole lens or the sensitive part of the lens. Whilst this implies that the accuracy of the model is not important for neutrons, it is probably a reflection of the low energy cut off of *Z* MeV for the secondary charged particles in “MCNPX” [264], where *Z* is the atomic number. This lack of sensitivity may hence demonstrate the inability of some Monte Carlo codes to accurately model energy deposition on the required scales for eye dosimetry.

 Models of cataractogenesis focus on smaller volumes, and in particular thinner regions, than in the eye model adopted by ICRP. Scoring doses in these parts of the lens will be important for accurate understanding of the mechanisms by which IR may induce cataracts. For example, calculation of the dose deposited in the epithelium, which is a single layer of cells, requires accurate radiation transport on a very small scale. Calculation of the absorbed dose due to photons must use precise transport of the secondary electrons, which even if the code being used is capable of doing this, may require great care to ensure that the best physics models are invoked. If they are, then calculation of the absorbed dose within cells or layers of cells is perfectly feasible [265,266], albeit without the option of experimental verification of the results. If calculating for higher LET particles, such as the secondaries from neutron exposures, the calculations will be more complex. A secondary proton with about 50 keV will traverse a cell, but transport of protons of such low energies is still not possible in most Monte Carlo codes.

 Computation power is still not sufficient to track individual electrons in even relatively small scale problems, and this issue is compounded by the poor interaction data available for electrons with energies lower than 10 keV; at very low energies, it becomes unrealistic to consider the electron as having a “range” because the paths become so complex, but a 10 keV electron travels about 25 µm in tissue, the diameter of 2-3 cells. Electrons of such low energies are important because experiments are likely to use, for example, 662 keV photons from 137Cs, but these will ultimately deposit dose via a huge number of sub-keV electrons generated via Compton scattering or photoelectric absorption, subsequent *e– - e–* scattering and various fluorescence and relaxation processes. However, some codes are beginning to make improvements at this scale and in this energy range [267–269]. These enhancements to the computational tools available offer the potential for modeling dose deposition at subcellular levels including DNA damage. In addition, further advances to model components will assist in accuracy of dosimetry going forward [270].

**7. Additional confounding factors**

*7.1. UV*

 Several epidemiological studies have shown that increased UV exposure (specifically UVB) is a significant risk factor for development of cortical cataracts and PSCs (and to a lesser extent, nuclear cataracts) [271]. UV radiation therefore represents a potential confounding factor when determining the effects of low doses of IR on the lens, particularly as UV exposure activates similar DNA repair pathways. For general cataract development, the Alienor study found significant associations between lifetime ambient UV exposure and cataract extraction surgery [272]. However, the authors of this work are only aware of a very small number of studies in which UV exposure (280-330 nm or UVB; at home or at work, using ambient levels as a proxy for amount of exposure) was evaluated as a potential determinant of cataractogenesis in individuals receiving IR exposure. Minamoto et al. proposed that UV radiation might explain the observed differences in cataract prevalence in Hiroshima and Nagasaki [273]. Chodick et al. [139] followed the incidence of cataract in US radiologic technologists over a protracted time. The median occupational IR dose to the lens in the cohort studied was estimated (by calculation) to be approximately 28 mGy. Individuals with the highest mean annual residential UV exposure at 13 years of age had a higher cataract risk, but, in common with other studies which do not directly measure doses, and thus potentially due to uncertain dosimetry, their comparison did not reach statistical significance after adjustment for IR dose received.

More studies are needed to determine whether UV and IR induce cataracts via the same mechanisms, and if the action of these two radiation sources is synergistic or merely additive. In the future, such data may come from population background studies, or studies of pilot or astronaut populations. However, for astronaut population studies within the past decade, the influence of exposure to non-spaceflight UV and IR exposures, as well as space acquired UV doses, was not controlled [274] and thus there may be many additional confounding factors that are applicable for these populations.

*7.2. Diet*

 It has long been recognized that nutritional deficiencies may lead to cataracts [275], but nutrition may be a confounding factor in the evaluation of cataracts induced after exposure to low doses of IR. This is because antioxidants, some of which may be found in common foods, have been shown to delay the appearance of opacities if administered to animals in their diets prior to irradiation ([276] and references therein). Differences in human diets (which may be rich or deficient in antioxidants) may therefore constitute a confounding factor in epidemiological studies of the effects of IR, especially for low doses. Indeed, nutritional intake has been adjusted for in a small number of epidemiological studies, including the NASA Study of Cataract in Astronauts [277,278].

# *7.3. Age and gender differences in radiation cataractogenesis*

 Most experimental, clinical and epidemiological data suggest that age and gender are determinants for IR cataractogenesis (see [279] and references therein). The age response for cataractogenesis varies with LET and dose, but in ways that are not entirely intuitive. For example, older rats exposed to low LET 60Co γ-rays showed a higher rate of increase in the development of cataracts compared to younger rats, but the latent period was greatly reduced and cataract incidence was much greater in younger rats. Furthermore, the progression rate of cataractogenesis was much greater in the irradiated eyes of older rats compared to younger rats exposed to high LET iron ions, and the latent period was reduced and incidence was enhanced in older rats. Regarding dose, older animals show a greater sensitivity for cataractogenesis after low dose irradiation, while higher dose favors progression of lesions in younger animals [200].

 Cataract incidence was significantly lower in female rats when compared to males exposed to low LET IR, but there was no difference in the rate of progression. Conversely, male rats had a lower incidence of cataracts compared to females exposed to high LET IR, and a lower rate of progression of cataracts [280]. Furthermore, while females are known to have a higher background risk of cataracts than males [281] and gender is usually accounted for in the human IR cataract studies (e.g. [139]), no significant effect of gender has been identified in the epidemiological literature.

*7.4. Hormonal modulation of radiation cataracts*

 Gender and age can modify the pattern of IR cataractogenesis [281], and hormonal modulation could explain some of the differences in sensitivity between the old and young, and males and females [279,282,283]. Interestingly though, IR cataractogenesis may be selectively modulated by exogenous hormone treatment if administered at different times before or after irradiation. Estrogen administered prior to irradiation was shown to potentiate cataractogenesis in ovariectomized rats, while treatment after irradiation provided a dramatic sparing effect [284].

 The evidence in rats for a role of hormone modulation is mirrored in frogs. In one study, irradiated-hypophysectomized (mitosis halted) frogs failed to develop opacities, while those with pituitary hormonal replacement (mitosis reinstated) developed cataracts following irradiation [14,129].

*7.5. Other*

 There are a number of additional factors that contribute to cataractogenesis. These include race [94,95], genetic and/or health status [67,88,215,221,226,285], trauma [286], and the use of medications including steroid treatment [287,288]. It is not possible to review the related literature in full here, but many of these factors may act in parallel or in combination with IR mechanisms in the initiation or formation of IR induced cataracts.

**8. Discussion**

 *8.1. Current status of knowledge and research gaps*

The IR response of the lens is a topic of current interest, not least due to the recent conclusions of ICRP that the lens is much more radiosensitive than previously thought [12]. ICRP recommendations that the threshold for IR induced vision impairing cataracts be considered to be 0.5 Gy, leading to an occupational dose limit of 20 mSv per year, have sparked a number of studies into the mechanisms of IR cataract. This review has considered papers from a large number of sources to bring together the available evidence for how IR initiates or promotes formation of cataracts.

 Fig. 2 summarizes the current status of knowledge on this subject from all the available literature. The lens biology is generally well understood, which in turn informs studies of the perturbation of normal function following IR exposure. *In vitro* and *in vivo* studies considered in this review have yielded information on a number of pathways induced by IR in human LECs, many of which warrant further investigation. These include the role of DNA damage and repair/misrepair processes, damage to the ECM, proteins or membrane lipids, changes in gene and protein expression leading to altered protein functions and morphological changes in LECs and protein fibers, the role of oxidation throughout and the role of NTEs and intercellular communication of factors including glucose transport and Ca2+ signaling. In addition, pathways of senescence, EMT and cell mobility/migration may also play a role. Finally, the importance of the geographical location of mechanistic actions in the lens remains unclear: for instance, the relative impact of loss of antioxidant capacity in the zones of the LECs compared to the mature LFCs.

 Animal models have allowed the evidence base to be further expanded to demonstrate a direct relationship between dose and cataract. Several studies have demonstrated the potential role of genetic background and strain dependence in sensitivity to IR cataract. The background rate of all cataracts dramatically increases with age [289]. Animal studies have demonstrated that age at exposure could indeed be a very important factor. However, the animal data must be considered in the light of the fact that the classification of cataracts is still largely qualitative and subjective, with several different classification methodologies in use, so studies are not always directly comparable.

 The most important endpoint for study of IR cataract is the evaluation of clinically relevant cataracts in exposed human populations. While there have been a relatively large number of informative epidemiological studies, very few of these provide useful information regarding the mechanistic processes. Nevertheless, the evidence thus far supports the role of oxidative damage, DNA damage and response pathways including methylation and gene expression following DNA damage, telomeric effects, genetic background and intracellular communication. Data on UV lens effects in human populations could also be informative. Most importantly, however, the human data provide evidence that low doses of IR cause cataracts.

<Figure 3 here>

*8.2. Future research perspectives*

Fig. 3 summarises some of the open research questions and, together with Fig. 2, illustrates the current information and favored hypotheses from the different levels of studies considered in this review. Figs. 2 and 3 highlight that, despite a relatively large amount of recent research in this field, there are a number of gaps in our knowledge and understanding, so the precise mechanisms remain largely unknown. Further, while there is information regarding a number of potential steps in the process of radiation cataractogenesis, it is important to note that there are still no hypotheses for complete mechanistic pathways. For example, although DNA damage and repair/misrepair have been observed in human and animal LECs (both in primary cultures and in established cell lines) following IR exposure, with observed dependence on dose [107] and IR quality [116]; DNA repair pathways are involved in maintaining the transparency of the lens [77]; impaired DNA damage repair also enhances the progression of spontaneous cataractogenesis regardless of IR exposure [76], and DNA damage is also known to be present in cataractous human lenses with nominal age-related and genetic causes [163,211]. Thus, the link between such damage and opacification is still unclear. Certainly, Figs. 2 and 3 show that there are a number of mechanistic strands common to all experimental models; however, it is clear that a combination of *in vitro*, *in vivo* and epidemiological human studies will be needed to answer the remaining research questions.

 When primary cultures or established cell lines demonstrate the same characteristics as LECs *in vivo*, *in vitro* studies can offer a useful tool to identify mechanistic targets (altered proliferation, differentiation etc.) as well as studying commonalities among cataractogenesis initiating agents such as steroids. They also give the ability to study physical properties such as IR quality, dose and dose rate. Many of these mechanisms can be validated using animal models for *in vivo* relevance. Useful tools for the investigation of genetic backgrounds include mouse models deficient in certain pathways such as DSB repair offering susceptibility to cataract induction. Indeed, animal models are needed because of the difficulties in accurate dose determination for large scale and/or retrospective epidemiological studies and the practical difficulties in obtaining exposed human lenses or extracting lenses that can then be exposed. Thus, mechanistic detail can only be thoroughly investigated using *in vitro* and animal studies. Finally, a number of existing human cohorts can help estimate risk with support from animal models. These cohorts also give a range of different exposure scenarios, such as acute (A-bomb survivors) and protracted (clinical or occupational) which are difficult to replicate experimentally.

The lens constitutes an excellent model to evaluate IR damage in general whilst highlighting particular aspects of the biological response to low dose IR. The lens continues to grow through life. The lack of removal of dead cells, the avascular nature of the lens, and the fact that it is comprised in a thick basement membrane make the lens a relative isolated tissue, which enables timed measurement of local cellular and molecular changes following IR. As such, it has been suggested that the lens IR effects might act as a “bioindicator” of systemic individual IR susceptibility [290]. Indeed, the lens may reveal aspects of the IR response of other tissues; certainly the relative resistance will be of interest. Following the reported findings that irradiated cells release certain factors, including cytokines [291], studies to monitor blood biochemical changes following IR exposure might be informative in terms of identifying global compromization of antioxidant defences [292] that could then be transferred to the lens.

 There is indeed a lot to be learned about NTEs in cataract for which formation of a number of *in vitro* models would be suitable. For instance, it is possible that GJ channels could transfer genotoxic signals such as long-lived radicals expanding the sensitive area required for cataract induction. *In vitro* studies looking at RIBEs and GJ inhibition are thus potentially interesting investigative avenues. In addition, understanding of the role of genetic background should lead to a fuller comprehension of the importance of individual sensitivities (the companion paper in this issue further deals with the individual sensitivity issue [293]).

In the past, ICRP recommended different thresholds depending on exposure conditions (acute or fractionated/protracted/chronic) and levels of the effects (minor opacities or vision impairing cataracts) [6], but now recommends a single threshold dose of 0.5 Gy assuming that minor opacities are formed and progress to vision impairing cataracts independent of rate of dose delivery [12]. Thus, one of key questions that need to be further addressed is whether there is any dose rate effect. Intriguing in this regard is the recent epidemiological evidence demonstrating a significantly increased risk for PSCs in the residents of the natural high background area (Yangiian, China) [294]. The threshold for protracted exposures in Chernobyl cleanup workers is not higher than the threshold for acute exposure in A-bomb survivors [12,295–298]. The cataractogenic dose in the Yangiian studies is yet to be reported but should be much lower than that in the Chernobyl studies. Altogether, these suggest “inverse” dose rate effect.

An additional important area of investigation will be the issue of latency period – there is a distinct lack of information regarding the progression and observed lengthy latency period from the initial IR insult to the appearance of observable/clinically relevant cataracts [28], and the apparent inverse relationship with exposure dose and time to manifestation is not at all understood. NTEs may have a role to play here; particularly RIBE.

One problem with studies to date is that it is very difficult to monitor cellular and molecular alterations with time following IR exposure (as responses differ *in vitro*). Identifying a valid marker for *in vitro* cataract analysis, whether it will be a molecular phenotype or morphology changes, will be key. This would ultimately offer *in vitro* studies more weight and help identify important changes post irradiation that correlate with this *in vitro* marker, rather than changes that are “incidental to exposure.

Further development of non-invasive longitudinal monitoring techniques will be crucial here; however, investigating dynamic changes in the lens equator will particularly challenging. The age effect itself requires further analysis. For instance, it has been suggested that the elderly might be more susceptible to IR induced cataracts because their lens epithelium is less well organized [5], although the opposite has also been observed in the mouse [200].

 There is also a need for new technologies adapted to the lens system in small animal models in order to be able to carry out objective assessment of specific endpoints. Indeed, going forward, it is likely that experiments attempting to elucidate mechanisms of cataractogenesis will not be performed on humans. Hence, interpretation of the results will not be greatly aided by modeling of dose deposition in human phantoms or models of the human eye. Where smaller animals are used, the scales of the problem will be reduced, which will pose added problems for Monte Carlo calculations. It will also alter the nature of the IR field to which the lens is exposed; the energy threshold for electron doses in the lens will be much lower in smaller eyes. Ideally, such calculations will be performed in non-human phantoms or eye models. In this regard, a realistic model of the mouse eye could be important, though experiments could be performed on other animals, for instance, zebrafish [299]. A mathematical model could be produced by a simple rescaling of the ICRP model of the human eye, or from appropriate imaging. Non-human phantoms are being developed, as a response to ICRP recommendations on protection of the environment [300,301], but those models are not intended for protection against cataract induction. Because ethical issues over the dose of IR received by the animal that is being imaged are different compared to humans, some very high resolution models have been produced, with, for example, 50 million voxels in a model of a crab [302] (c.f. approximately 2 and 4 million voxels in the much larger ICRP human reference male and female phantoms [242]). So, it is possible that µ-CT images of a small animal could provide the resolution needed for lens dosimetry. However, this expanding field of interest includes a reference rat rather than mouse [300], though voxelized mouse phantoms exist [303,304].

*8.3. Implications and wider impact*

 ICRP considers IR induced cataracts as a deterministic effect with a threshold of 0.5 Gy for vision impairing cataracts irrespective of the rate of dose delivery [12], so that <1% of the exposed population are likely to develop IR induced cataracts with exposure doses <0.5 Gy. However, much of the mechanistic data indicate that cataracts do not really conform to the traditional definition of a deterministic effect or tissue reaction, which is defined as “injury in populations of cells, characterized by a threshold dose and an increase in the severity of the reaction as the dose is increased further.” [12]. Moreover, ICRP reports that “in some cases, these effects are modifiable by post-irradiation procedures including biological response modifiers.” [12]. The evidence suggests that the latter statement certainly applies for cataracts; however, there is also evidence that cataracts may be more stochastic in nature: in particular, the studies on genetic background (section 4) and the data suggesting that oxidative stress and DNA damage could be involved in cataractogenesis processes (section 3). Nevertheless, for radiation protection purposes, the classification of IR cataract as a deterministic effect remains in place. Indeed, radiation protection authorities can only ever make the best possible judgements regarding dose limits with the current information available. However, it is important to note that a fuller understanding of IR cataractogenesis may yet demonstrate that IR induced cataracts are indeed stochastic in nature. Therefore, a better understanding of the mechanisms of IR cataractogenesis is crucial to ensure that the most appropriate judgements are applied. Furthermore, a more complete mechanistic understanding might have important implications for the development of personalized medicine in years to come.

It is also interesting to note that there have been a number of promising potential countermeasures developed in the last few years, including a pharmacological α-crystallin chaperone which partially restored transparency in cataract models [141] and l**anosterol which reversed protein aggregation in cataracts in dogs and rabbits [305,306]; however, the relevance for IR induced cataracts *in vivo* in needs to be further considered.** It has been suggested that dietary radioprotectors might also be preservative against cataract development for astronauts even when taken after exposure [307], although this has only been tested in mice to date. Most recently, a successful technique has also been demonstrated for lens regeneration relying on endogenous stems cells [308]. However, such advances are still likely to be several years away from clinical use. Furthermore, the general risk of IR cataract within the population increases with increasing use of IR in medical and occupational settings. Despite increasing awareness of general radiation protection needs, the annual medical IR exposure of the US population increased six fold between 1982 and 2006 [57], and thus IR cataract is likely an increasing public health problem, rather than the reverse. Nevertheless, IR brings huge benefits to society. As such, further research on this important topic is justified in order to ensure adequate and appropriate radiation protection can be put into place.

 In conclusion, it is clear that normal lens biology, morphology and physiology are well understood, and many of the general opacification processes that have relevance for IR induced cataracts are also well characterized, particularly for age-related cataracts. In terms of the research to date, evidence from *in vitro* studies gives information about the steps in the chain; however, the relevance for cataracts in human populations must be validated in human lenses where possible and/or appropriate animal models otherwise. However, despite the recent advances detailed in this review, further study of the role of low dose IR in cataractogenesis is clearly needed, and we here recommend that particular attention be now paid to mechanisms of low dose IR cataract induction, the potential dose rate effect, and further development of accurate dosimetry techniques.

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**Conflict of interest statement**

The authors declare that there are no conflicts of interest.

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**Figure legends**

**Fig. 1.** Protein fiber and cellular organization within the lens. (A) The lens is formed from a single cell layer of lens epithelial cells (LECs) that covers the anterior portion of the lens. These LECs are in different stages of the cell cycle [32,78]. The cells in the central region are mostly quiescent; meanwhile the proliferating cells are largely confined to the germinative zone (GZ) in the equator of the lens. After division, LECs migrate to the transitional zone (TZ), situated immediately adjacent to the GZ and most distal to the anterior pole. In the TZ, LECs begin differentiation to form lens fiber cells (LFCs) that comprise the bulk of the lens mass. They enter the body of the lens via the meridional rows (MRs), adopting an hexagonal cross sectional profile, offset from their immediate neighbors by a half cell width to deliver the most efficient cell-cell packing arrangement that is perpetuated into the lens body as LECs continue their differentiation and maturation process into LFCs [29,32,78,100]. Any changes to the pattern of LEC proliferation or migration has the potential to affect their differentiation, translating into morphological and cell packing changes that can compromise lens transparency and its function [309–312]. LFCs are derived from LECs, and form the major component of the lens. LFCs become highly elongated and, depending on the size of the lens, they can be millimeters in length, possessing a highly accentuated aspect ratio because of the ~15 µm cross section dimension. LFCs pack in concentric layers, with youngest cells at the lens equator. The apical ends oppose the apical surfaces of the apposed LECs, whilst the basal ends are attached to the posterior surface of the lens capsule maintaining polarity for these LFCs. As the fibers elongate, their apical and basal ends converge at the anterior and posterior lens poles. (B) The lens sits in the anterior portion of the eye where it focuses light onto the retina to create a sharp image (top). However, when a cataract develops, the transmission of light is either blocked or not focused correctly (bottom), creating a distorted image (section 2.5). (C) Example of lens fiber sutures as viewed from the posterior pole of the lens in the healthy lens compared to an example of an abnormal, cataractous lens: where the LFC tips contact each other, anatomically visible structures called sutures form [313]. These can be either point, or line or star shaped suture patterns dependent upon the species. These sutures cause localized disruption to the light refraction, but in primates, suture organization has been optimized so that the disruption to its refractive properties is uniform across the lens [314]. This LFC packing and suture patterns are also age dependent, but in all lenses, the oldest LFCs are located in the core of the lens, and the younger ones are close to the MR [30,31].

**Fig. 2.** Representation of IR responses characterized in human and animal lens epithelial cells or cell lines, *in vivo* animal studies and limited information from human studies. Cx, connexin; ECM, extracellular matrix; FGF, fibroblast growth factor; IR, ionizing radiation; LEC, lens epithelial cell.

**Fig. 3.** Open questions for cellular, animal and human studies, and some potential areas of future research. IR, ionizing radiation; RT, radiotherapy; UVB, ultraviolet B.