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Age-Related Human Nuclear Cataract. A Condition due to Inexorable Protein Deterioration

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Abstract

Nuclear cataract stems from the inexorable breakdown of long-lived macromolecules in the human lens. Although this realization is quite new, and the details of several events need to be determined, the overall framework is now reasonably clear. Racemisation, deamidation and truncation are the main drivers of protein denaturation, although understanding these processes leads to a conclusion that the prospects for reversing lens opacification are remote. Since age-related cataract appears to be inevitable, future strategies for slowing cataract formation may depend on a detailed examination of people who retain clear lenses into their eighth and ninth decades.

Keywords: Cataract; Lens; Age; Human; Racemisation; Deamidation; Truncation

Introduction

A major advance in ophthalmology in recent years has been elucidation of the sequence of steps involved in human age-related nuclear cataract. Many years of research can be neatly encapsulated in just one sentence. Nuclear cataract is due to the decomposition of long-lived macromolecules in the human lens.

It is important to recognize that nuclear cataract, which is typically colored and affects primarily the centre of the lens, can be differentiated from two other, less common types of human cataract: posterior subcapsular (PSC) and cortical. Much less is known about the etiology of the cataracts that involve the outer part of the lens, but it is thought that cortical cataract results from osmotic/electrolyte abnormalities, and PSC could originate from damage to epithelial cells that undergo mitosis at the lens germinative zone.

Animal Experiments

Traditionally cataract research has involved two strands: one directed towards an examination of authentic human cataract lenses, and another avenue sought to study animal models with a view to extrapolating the data into the human realm.

A major difficulty with animal experiments is that humans are not large hairless rodents. Human lenses are different in many key ways from those of laboratory animals [1]. In addition, a plethora of agents can induce cataract. Mutations, diet, radiation, drugs and toxic chemicals can all lead to lens opacification.

Underpinning the laboratory experiments on rodents (typically) and their lenses was an article of faith (expounded in manuscripts and grant applications) that if the mechanism for the particular animal model were deduced, then the data could be extrapolated to human age-related cataract. Unfortunately, in my view, the volumes of data accumulated did not contribute significantly to our understanding of human nuclear cataract, which is almost totally age-related. Why this is so, will hopefully become evident in the rest of this article.

It is only in retrospect that it can be appreciated that a focus on animal model systems for uncovering the mechanism of human cataract yielded little data that were useful for understanding the human condition. It could even be regarded as being counter-productive, because it diverted limited resources from a direct study of human lenses. This topic remains controversial and will no doubt be the subject of future study sessions.

There are a few exceptions where animal data could be useful for understanding processes at work in human cataract. Experiments by Giblin's group employed hyperbaric oxygen to induce cataract in guinea pig lenses and whole animals [2]. These experiments highlighted the marked differences in the response of the lens nucleus and cortex to oxygen. In this regard they mirror the results obtained from human nuclear cataract lenses [3]. It is likely that the potential usefulness of these experiments stems from the fact that a physiologically-relevant oxidant was chosen to induce lens opacification and experiments were conducted painstakingly using low exposure over a long time period [4]. Intriguingly oxygen exposure leads to truncation of aquaporin 0 and this replicates a feature that is observed in human lenses.

It is obvious from an historical perspective that some seminal work was undertaken on human lenses. In particular, great strides were made in the 1960s and 1970s at the Nuffield laboratory in Oxford, U.K. The development of the Pirie classification system for grading cataract lenses provided one of the most important frameworks, since it allowed the clinical presentation to be linked with biochemical analyses. The correlation was often remarkable [5] especially considering that the cataract classification system is largely subjective. Unfortunately for those poor souls who toil to understand the etiology of human cataract, advances in cataract extraction methods mean that it is now almost impossible to source intact cataract lenses.

Root Cause: The Gradual Deterioration of Macromolecules

A fundamental breakthrough in understanding human cataract came with recognition of the extent, and progression, of age-related

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degradation of the lifelong macromolecules that make up human lenses. It had been thought for many years that turnover of proteins in the lens was minimal, or non-existent, however data to confirm this did not eventuate until 14-C measurements were undertaken [6]. This novel technique compares the content of 14-C within macromolecules, to atmospheric levels of the radioisotope that resulted from nuclear testing [7]. If levels in a particular molecule match those at the time of birth, then there has been no turnover.

It may be imagined that in a metabolically quiescent tissue, with high antioxidant levels and low oxygen tension, that macromolecules could potentially be stable over a long time period. Limited early experiments suggested that this may not be the case and that some degradation of proteins from normal lenses was observed with age. These changes were however, most often examined in isolation i.e. one site in one purified protein, so it was difficult to evaluate the overall modification in the context of the human lifespan.

Many changes occur to lens proteins as we age e.g. [8-11] however it is not the purpose of this article to review this considerable literature, but to focus more on the most recent developments that pertain to cataract etiology. Some of these will be discussed later in this article. The impact of a range of PTMs on the properties of proteins in the lens is difficult to model, but it is clear that as they accumulate, aged human lens proteins denature. One result is that their solubility alters.

Increase in Insoluble Protein and Loss of Soluble Proteins

There is an almost linear increase in insoluble protein in the human lens over our lifespan. Much of this is alpha-crystallin. It is probable that this conversion of formerly soluble crystallin to insoluble protein is due to the unfolding of polypeptides and the binding of the chaperone, alpha-crystallin to form high molecular weight aggregates. Consistent with this picture is the fact that soluble alpha-crystallin decreases to negligible levels by about age 40 [12]. Using antibodies it has been found recently that substantial conversion of soluble to insoluble γS-crystallin takes place in the first three decades of life [13].

What Processes are Responsible for Age-related Crystallin Unfolding?

The major quantitative changes to crystallins are deamidation, racemization and cleavage. These have been discussed in more detail elsewhere [14]. Several patterns can be discerned. Firstly, some crystallins are more susceptible to modification than others. The α-crystallins, and in particular αA-crystallin, are prone to all of the three major posttranslational modifications (PTMs) listed above. Others, such as the γ-crystallins and βB2-crystallin, appear to be considerably more resistant [15]. One feature that could be responsible is the compactness of protein three-dimensional structure and the degree of unstructured polypeptide within it [16]. For example, γS-crystallin is more predisposed to these age-related PTMs than the more compact γC and γD-crystallins, and it is known that γS-crystallin is a more open structure and as a consequence it elutes earlier than the other γ-crystallins on gel filtration.

Non-enzymatic cleavage of peptide bonds at Ser [17,18] and Asn [19] residues is responsible for another class of lens PTMs. Little work has been undertaken to discover the effect of these peptide bond scissions on 3D structure of the shortened proteins. Of course, truncation in the internal part of the protein is very likely to completely disrupt the structure. The flexible terminal extensions appear to be more susceptible to spontaneous peptide bond splitting. For example, scission at each of the Ser residues in the flexible C-terminal tail of γA-crystallin has been reported [20]. Recently it has been discovered that the small peptides that result from non-enzymatic cleavage of crystallins may themselves have biological activity. For example, peptides derived from α-crystallin seem to promote protein aggregation [21] and a peptide formed by the age-dependent cleavage of the C-terminus of at Serine γS-crystallin inserts into fibre cell membranes and alters their permeability [13].

Some Common PTMs are Absent from Lens Proteins

One notable feature of the lens is the PTMs that are not observed. More correctly, some "expected" PTMs are found at levels that are orders of magnitude lower than those summarized above for racemization and deamidation. The trace PTMs of note in this regard result from protein modification by monosaccharides and their metabolites [22,23]. When one considers that glucose is the main fuel of the lens, and is present over a period of decades at mM concentrations, the levels of characteristic glycation products such as carboxymethyl and carboxyethyl Lys are truly miniscule [24]. One novel PTM that does contribute to age-related lens coloration appears to be the result of lysine (Lys) residue modification by breakdown products of ascorbate [25,26]. This antioxidant, and its tricarbonyl oxidized partner, dehydroascorbate, are more prone than monosaccharides to decompose under biological conditions. The reason for the lack of protein modification by sugars and their metabolites is not clear but is likely the consequence of high glutathione levels that bathe the crystallins [27]. Another possible factor is the high rate of metabolic conversion of unstable pyruvate to lactate that then diffuses from the lens. Despite the high levels of glutathione in the lens, that acts to intercept potentially damaging molecules, some reactive chemicals such as S-adenosyl methionine do react with crystallins over time, and the levels of methylation can reach high levels [28] . This fact argues against crystallin structure and amino acid availability being solely responsible for the lack of reactivity with sugars.

Another PTM that is almost absent from normal human lenses is oxidation [29,30] and this surprises many people who are aware of proteomic work where oxidized peptides have apparently been identified from old crystallins. It is very easy to generate artefactual oxidation of some residues during sample handling.

It should be emphasized that the composition of membranes of human fibre cells, as well as proteins, alters dramatically with age [31,32]. The implications of this phospholipid alteration may well be substantial, for example in the genesis of the lens barrier, but will not be covered in this review.

Proteomics Uncovers the Basis for Cataract

The advent of modern proteomic techniques allowed scientists to gain data on the precise location, and extent, of the changes to individual lens proteins as a function of human age. Importantly individual crystallins could be compared contemporaneously from the one lens and the data compared to those from other lenses.

These studies have revealed how extensive the changes to proteins in normal lenses are as we age. Indeed a remarkable feature is that, considering the many sites involved, and the magnitude of the changes at so many sites, that in most cases adult human lenses remain transparent! This is particularly so since these modifications are cumulative.

Mutations can Act as a Template

Most of the research data on human lens aging and cataract are necessarily correlative in nature, in the sense that it is not possible to manipulate the genome, or modify the sequences of proteins in human lenses, to mimic the documented changes and then to observe an effect on lens transparency.

One powerful tool that is available at our disposal is naturally-occurring mutations in the population that lead to cataract. The molecular bases of many of these have now been characterized. Most involve single amino acid substitutions.

A number of cataractogenic mutations can be traced to a conservative substitution in just one crystallin. This illustrates clearly that very minor changes to a single protein can be sufficient to cause human lens opacification. On this basis one might predict that even relatively slight changes to crystallins over time could be sufficient to transform the transparent lens into one that no longer properly transmits light.

Some Modifications are More Important for Transparency than Others

Once it had been appreciated that the changes with age to both proteins and phospholipids in normal lenses were large, other key questions emerged:

"Could changes to one, or possibly just a few key macromolecules be sufficient to induce cataract formation? If so, which ones?"

One way to answer this is to compare cataract lenses with age-matched normal lenses. Prior to the commencement of these studies some basic tenets were formulated. If modifications at a particular site were extensive, yet the degree and time course of appearance did not differ significantly between cataract and normal lenses, then it could be concluded that they were unlikely to be involved in cataract formation.

If, on the other hand, one site showed a consistently different type or degree of modification in cataract lens proteins when compared to age-matched normal lenses, then it could be considered to be potentially cataractogenic. This would be especially so if the degree of posttranslational modification were greater in all of the cataract lenses across the age range.

Experimental Approach is Critical

It is worth recognizing a procedural issue at this point. A potential problem will arise if the experimental strategy to uncover protein differences that may be cataractogenic, involves a comparison of proteins purified from normal and cataract lenses. This is so, because multiple soluble and insoluble forms of each polypeptide exist in aged lenses and thus any isolation strategy may well selectively enrich one form in one lens type, thus rendering proper comparisons invalid. The fact that nuclear cataract lenses contain more insoluble and oxidized polypeptides will further confound any comparison. To obviate this, whole lenses, or better still, nuclei from cataract and age-matched normal lenses, should be analyzed without prior fractionation. Data are now available from such analyses and will be discussed in the next section.

Some Sites Change Substantially with Age but do not Appear to be Cataractogenic

To illustrate this using one example where a large modification occurs with age but does not appear to be implicated in cataract, Asp 151 in αA-crystallin can be used [33,34]. This amino acid racemises early in life, such that half of the original L-Asp has been lost by the teenage years. Despite this very substantial modification, there is no significant difference in the degree of isomerization for any of the Asp isomers at residue 151 between cataract and normal age-matched lenses across the age range.

Some Sites may be Cataractogenic

A remarkable very recent discovery is that some sites in crystallins may be cataractogenic. The definition of "cataractogenic" in this sense is that the levels of modification of a particular protein at one site, are consistently greater in cataract lenses than in age-matched normal lenses.

So far five sites, in two proteins have been discovered that can be deemed cataractogenic. These are Asn 76 and Asn 14 in γS-crystallin [33], Asp 58 in αA-crystallin [35] and Ser 59 and Ser 62 in αA-crystallin [36]. All of these are localized within unstructured regions of the two crystallins, with Asp 58, Ser 59 and Ser 62 being from the same sequence of the protein that is adjacent to the "α-crystallin domain". Interestingly only the level of conversion of Asp 58 to the most disruptive isomer, D-isoAsp, differed significantly between the cataract and the normal lenses. Of the four possible Asp isomers formed from the succimide-mediated isomerization of L-Asp [37], D-isoAsp is likely to cause most alteration in protein structure because D-isoAsp is not only racemised, but an additional methylene group has been inserted in the polypeptide chain.

Changes Occur in Young Lenses

As will become apparent in this article, the accumulation of PTMs in proteins is becoming recognized as the probable basis for human age-related nuclear cataract.

One question then arises; "When do these changes begin?"

Several scenarios could be imagined. For example, there could be little measurable change in proteins in the first few decades of life and then PTMs may accumulate rapidly just prior to lens opacification; typically in the sixth and seventh decades. Alternatively PTMs could accumulate gradually throughout life. It turns out, based on time courses of crystallin modification that the latter sequence is the more accurate one.

Changes can be detected in young lenses. Intriguingly the rate of increase of some modifications occurs most rapidly in the first 15 years of life. This is true for racemization of Asp, Asn and Gln [16,33,38] as well as the cleavage of aquaporin 0 [39]. Why this should be the case is not known, but presumably reflects changes to more susceptible sites in some lens proteins. Since teenage lenses are almost always beautifully transparent, it can be concluded that this extent of PTM does not adversely affect the optical properties of the tissue.

Time Courses Differ from One Lens Protein to Another

Although there is a trend for every PTM elucidated thus far to increase in extent with age, two significant features have emerged from recent examinations of lens crystallins. Firstly, a particular amino acid residue (e.g. Asn) at one site has a time course that differs from that of the same amino acid at other positions within the one protein. Secondly not all crystallins are equally affected by the ravages of time. For example, βB2-crystallin seems to be relatively unchanged over our lifespan, whereas αA-crystallin and γS-crystallin undergo very large changes.

αA-crystallin serves as a good example to illustrate the age-dependent differences at geographic sites. Asp 151 isomerises and racemises extensively in the first decade of life, so that approximately two thirds of all L-Asp in this position has been converted to a mixture of D-isoAsp, L-isoAsp and D-Asp by age 10 [33]. Thereafter values change very little up till the tenth decade. Asp 58 behaves in a similar manner: approximately one half of the L-Asp at this site has been converted to the other Asp isomers by teen-age years. By contrast Asp residues in structured regions of αA-crystallin remain largely as they were created i.e. as L-Asp.

A different time course of formation is evident for D-Ser in αA-crystallin. At residues 59 and 62, racemisation takes place almost linearly over time [36]. Cleavage at Ser residues in the C-terminal tail of αA-crystallin is another event that takes place predominantly in the first decade. In the case of γS crystallin, the time course for Gln deamidation [16] is different from each of the αA crystallin PTMs.

How should we interpret such data? One possibility is that once a sufficiently high level of PTM accrues at one particular cataractogenic site then that lens becomes compromised. An alternative view is that a combination of PTMs at several cataractogenic sites is required and that once several of these reach beyond a certain threshold that lens opacification takes place. Both hypotheses could be valid.

The Consequences of Gradual Change become Apparent at Middle Age

As summarized briefly above, disparate modifications take place to each lens protein over our life span. Every one is likely to have some impact on tertiary structure inducing some degree of protein unfolding and eventually the modified protein may become insoluble. Analysis of lens insoluble protein indicates that insolubilisation occurs as a chaperone complex with α-crystallin. In the case of αA and B-crystallins their extensive PTMs with age will compromise their ability to act as molecular chaperones. It appears that high molecular weight aggregates, rather than amyloid, are formed from the denatured crystallins in older lenses despite the fact that crystallins do have the potential for conversion to amyloid fibrils [40].

In terms of relative time courses, the information on the insolubilisation of individual polypeptides is limited. In the case of γS-crystallin, a significant amount of the intact protein appears to become insoluble before age 20 whereas other modifications, such as peptide bond cleavage, are more prominent in middle age [13]. As noted earlier, soluble α-crystallin is undetectable in the human lens nucleus after middle age.

Despite the accrual of extensive changes at many sites in a number of different proteins, remarkably, in the vast majority of people, the lens remains transparent past middle age. This is astounding considering, as noted earlier, that small changes via mutations in one crystallin can lead to complete lens opacification. How this feat is achieved in the normal lens is still unknown however suspicion is directed towards the ameliorating effects of the α-crystallins [41]. Direct experimental evidence is lacking. Circumstantial evidence for the importance of chaperones is that once they have all been totally used to form high molecular weight complexes (i.e. no free soluble alpha-crystallin remains in the nucleus) then major changes begin to be noticed in the lens itself. This occurs at middle age.

Most notably after age 40-50, proteins bind strongly to cell membranes [42,43]; the lens barrier develops [44] and cataract ensues.

Only Human Lenses will do for the Future

Issues associated with the use of animal models for human cataract have been discussed in greater detail elsewhere [45]. Primates, such as rhesus monkeys, may provide the closest model system, but their lifespan is significantly shorter than ours, and there are also significant differences in the lenses of the two species [46].

A realisation that the vast majority of animal models have little role to play in deciphering human age-related cataract, means that the future for cataract research, and potentially other human age-related research, is made much more difficult. Options and strategies are markedly restricted. Limitations of *Homo*-focused research will not be discussed in detail here, but can be summarized as: a lack of replicates, human individual variability, lack of control over, or the ability to manipulate, diet, genes and environment. To be added to this already daunting list is a lack of access to tissue, limited control over times of collection and storage, the amount of material available, the possibility of infectious disease and more stringent ethical hurdles. Lifespan, and the time for conditions to become apparent, are other key differences in comparing laboratory animals and humans. Age-related human cataract typically takes more than 50-60 years to develop. Little wonder then that animal studies blossomed while human research has declined in the past three decades.

One positive feature in the lens arena, which is lacking in some other age-related human research, for example that on the brain, is the availability of tissue. Donor eyes are an invaluable source of material and from them intact normal lenses of different ages can be sourced quite readily, at least in most Western nations.

Even so, some ages are much more difficult to obtain than others; in particular lenses younger than 20 years old.

Given the well-known variability of the human population it was perhaps surprising that the data on the extent of modification of individual crystallins has been so consistent [16,33,38]. At first this may seem at odds with the fact that some people get cataract at age 60, whereas others retain clear lenses into their 80s. Understanding why this is the case, seems to be one of the key questions to answer for future lens researchers.

Future Prospects

It is certainly not a novel observation to suggest that in the human breast, hope springs eternal. Particularly in the general media, news stories that focus on the prospect of a cure for any age-related disease unsurprisingly generate more interest, and positive sentiment, than those where the summary of years of research is that decline and demise are inevitable. Unfortunately, in the case of human age-related cataract, there appears to be no prospect of reversing cataract once it has formed using pharmacological treatment. Surgery is the only option.

This is not to say that intervention prior to lens opacification is unimaginable. Currently ophthalmic researchers have little, or no, idea why some individuals retain transparent lenses well into their ninth decade. This fact alone suggests that lifestyle modification, or future drug treatments, may allow many more individuals to continue to see into old age without the need for plastic lenses.

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