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Endoplasmic Reticulum Stress as a Primary Pathogenic Mechanism Leading to Age-Related Macular Degeneration

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Abstract

Age-related macular degeneration (AMD) is a multi-factorial disease and a leading cause of blindness. Proteomic and genetic data suggest that activation or de-repression of the alternate complement cascade of innate immunity is involved in end-stage disease. Several lines of evidence suggest that production of reactive oxygen species and chronic oxidative stress lead to protein and lipid modifications that initiate the complement cascade. Understanding the triggers of these pathogenic pathways and the site of the primary insult will be important for development of targeted therapeutics. Endoplasmic reticulum (ER) stress from misfolded mutant proteins and other sources are an important potential tributary mechanism. We propose that misfolded-protein-induced ER stress in the retinal-pigmented epithelium and/or choroid could lead to chronic oxidative stress, complement deregulation and AMD. Small molecules targeted to ER stress and oxidative stress could allow for a shift from disease treatment to disease prevention.

46.1 Age Related Macular Degeneration Is a Leading Cause of Vision Loss

AMD is the leading cause of visual impairment in the elderly (Javitt 2003; Klaver et al. 2001; Klein et al. 1992) affecting an estimated 10 million Americans and 50 million people worldwide. AMD is clinically heterogeneous and is diagnosed irrespective of visual acuity. Late AMD is described as either dry or wet depending on the absence or presence of choroidal neo-vascularization (penetration of the choroidal vasculature into the subretinal space). Most patients have dry AMD where focal degeneration of photoreceptors, RPE and choriocapillaris in the macula (together called geographic atrophy) impairs visual acuity over time. In contrast, in wet AMD there is often sudden and acute vision loss because of choroidal neo-vessels. The presence of wet and dry lesions within the same eye, the observations of dry AMD progressing to wet AMD, and the lack of significant differences in the frequency of risk alleles of predisposing genes between the two sub-groups support the notion that common mechanisms underlie all disease classes.

46.2 Oxidative Stress and Complement Activation are Common Pathways in End-Stage Disease

Important studies of human eyes showed components of the alternate complement pathway of innate immunity, and acute phase response proteins of the systemic arm of innate immunity, accumulated at the RPE/Bruch's membrane interface in AMD patients. These observations led to the hypothesis that activation/de-repression of the alternate complement pathway, and a smoldering inflammatory response, might be central to AMD pathogenesis

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(Crabb et al. 2002; Hageman et al. 2001). Genetic data support this contention, and loci containing genes encoding components of the alternate complement pathway are reproducibly associated with disease risk (Edwards et al. 2005; Hageman et al. 2005; Haines et al. 2005; Klein et al. 2005). Complement activation/de-repression appears to be a common end-stage pathway where other tributary mechanisms have converged. However, the cellular mechanisms preceding complement activation/de-repression are largely unknown. Unfortunately, identifying primary mechanisms for age-related diseases is often confounded by complex genetic interactions (Klaver et al. 1998) and a lifetime of variable exposure to environmental factors. Several factors are implicated in AMD pathogenesis, including high fat diet (Cho et al. 2001; Seddon et al. 2003), pathogen load (Kalayoglu et al. 2005), increased light exposure (Taylor et al. 1990), choroidal hypoperfusion (Friedman et al. 1995), and cigarette smoking (Klein et al. 1993). Importantly, these associations, and several other lines of evidence (Beatty et al. 2000; Decanini et al. 2007; Shen et al. 2007; Suzuki et al. 2007), strongly support a role for oxidative stress as a primary trigger of AMD pathogenesis.

Oxidative stress refers to the cellular or molecular damage caused by reactive oxygen species. In addition to abnormally oxidized proteins, lipids, and nucleic acids localized in drusen, much evidence supports an important role of oxidative stress in AMD pathogenesis. The retina is one of the highest oxygen-consuming tissues in the body, and the RPE is exposed to high levels of oxidative and photo-oxidative damage over a lifetime (Beatty et al. 2000). Increased production of reactive oxygen species is a proposed cellular insult from photo-oxidative damage. Lipofuscin (lipid-containing pigment granules associated with aging) and the lipofuscin retinoid fluorophore A2E (*N*-retinylidene-*N*-retinylethanolamine) are associated with AMD and are proposed to exert their harmful effects via production of reactive oxygen species and oxidative damage (Sparrow et al. 2002; Zhou et al. 2006). Despite the increasing threat of oxidative stress, ability of cells to detoxify reactive oxygen intermediates deteriorates with advancing age. Together, the deteriorating capacity to detoxify reactive oxygen species and the cumulative effects of oxidative stress are proposed to be a local insult contributing to disease (Beatty et al. 2000). This hypothesis is supported by studies suggesting that factors that increase oxidative stress exacerbate disease, while factors that ameliorate oxidative stress slow AMD progression (Bazan 2006; Group 2001; Tan et al. 2007). Finally, there is strong evidence for the importance of oxidative stress in vivo in AMD pathogenesis from several mouse mutant strains (Dong et al. 2009; Hollyfield et al. 2008; Imamura et al. 2006; Wong et al. 2007).

46.3 ER Stress and Oxidative Stress Interact

Protein folding leads to a net loss of reducing equivalents via disulfide bond formation. ER associated degradation (ERAD) is an important consumer of reducing equivalents because a large proportion of proteins (even under physiologic conditions) submit to degradation. Furthermore, protein secretion consumes cellular cysteine and depletes the cell of reducing equivalents. Thus, protein folding and subsequent degradation or secretion entails a net loss of reducing equivalents (Banhegyi et al. 2007). This is exacerbated when genetic mutations affect protein folding. Illegitimate disulfide bond formation, reduction/replacement by isomerization, and eventual ERAD all consume and further deplete cellular reducing equivalents. Oxidative stress in the ER contributes to endogenous peroxide generation in the cytosol and ER isomerases interact with glutathione that retro-translocates to the cytoplasm (Sevier and Kaiser 2008). Therefore, folding and secretion of proteins produces reactive oxygen species and illegitimate disulfide bond formation and subsequent ERAD of misfolded mutant proteins increases oxidative stress within cells (Sitia and Molteni 2004).

In normal protein folding, disulfide bonds are formed and broken as proteins are rearranged. Many chaperones are oxidoreductases with thiol groups that act as molecular switches for redox state and, thus, are sensitive to oxidative stress. In conditions of oxidative stress, the ratio of reduced/oxidized forms of chaperones available for normal protein folding is altered. Under these conditions there is illegitimate disulfide bond formation and stabilization of undesirable, intermediate conformations. Before misfolded proteins can be degraded, disulfide bonds must be reduced, however, excessive oxidation impedes disulfide bond reduction and inhibits ERAD. Thus, oxidative stress can inactivate chaperones, promote aberrant disulfide bond formation, promote stabilization of undesirable intermediates and inhibit degradation of misfolded proteins, which together cause ER stress (Banhegyi et al. 2007; Marciniak and Ron 2006).

46.4 ER and Oxidative Stress as Triggers for Inflammation and Disease

ER stress and oxidative stress lead to production of pro-inflammatory mediators including prostaglandins, leukotrines and tumor necrosis factor alpha (Hu et al. 2006) and de-repression of nuclear factor kappa B (Deng et al. 2004; Jiang et al. 2003). ER stress and oxidative stress can also activate systemic and local cascades directly implicated in AMD pathogenesis. C-reactive protein, and serum amyloid P are components of the systemic arm of the innate immune system, are localized to drusen, and are directly up-regulated by ER stress (Zhang et al. 2006). Moreover, local ER stress can lead to STAT3-dependent up regulation of vascular endothelial growth factor (a key molecular trigger for progression to CNV) (Zhang et al. 2006) and oxidative stress is shown in animal models to contribute to CNV (Dong et al. 2009).

There are relevant and important examples of ER/oxidative stress interacting and contributing to disease. Abnormally oxidized lipids in atherosclerotic plaques activate ER stress in endothelial cells and mediate a chronic inflammatory response. AMD and atherosclerosis are linked in epidemiological studies (Vingerling et al. 1995) and share risk factors including increased age, smoking, high fat diet and pathogen load. Both diseases have local extracellular deposits of abnormally oxidized molecules that precede chronic inflammation and there are striking similarities in the protein, lipid and cellular compositions between atherosclerotic plaques and drusen (de Boer et al. 2000; Hageman et al. 2001; 1999; Mullins et al. 2000; Suzuki et al. 2007). Importantly, mice immunized with an oxidization fragment of docosahexaenoic acid were shown to accumulate activated complement components in the outer retina and develop lesions resembling geographic atrophy (Hollyfield et al. 2008). This discovery and others (Wu et al. 2007) reveal a key link between oxidative damage, complement activation/de-repression, and retinal disease.

46.5 Future Experimental Approaches

Understanding the roles of ER stress and oxidative stress in AMD is important as these cellular processes represent pathways that could be targeted with small molecule therapeutics (Sauer et al. 2008). Studying retinal disease in mouse models with ER stress (via misfolded proteins or by other mechanisms) and in mutant animals that have misfolded proteins in combination with a reduced capacity to decrease oxidative stress (*Sod1*$^{-/-}$) or an impaired capacity to reduce ER stress (i.e. *Eif2a*^{S51A}) will contribute to testing this hypothesis. Bruch's membrane is an elaborate extracellular matrix. Extracellular matrix molecules are processed in the ER before secretion and represent strong candidate genes for ER stress-induced pathogenesis (Bateman et al. 2009). Conditionally expressed mutant alleles of extracellular matrix molecules can genetically dissect the relative contributions of RPE and choroid in the primary pathogenesis of disease. Understanding the primary location

of the insult will determine necessary properties of potential therapeutics targeted at this mechanism (Fig. 46.1).

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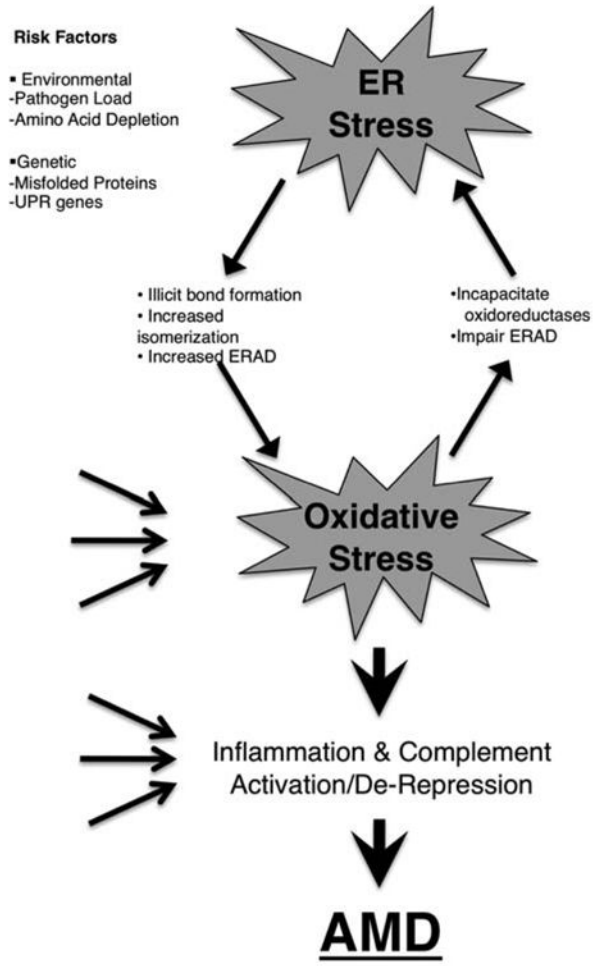

Fig. 46.1.

Evidence supports that oxidative stress and complement activation/de-repression are common end stage pathways in AMD and each pathway is likely to have several genetic and environmental factors influencing it. We propose that ER stress, caused by misfolded proteins or other insults could be a primary tributary mechanism leading to initiation of AMD pathogenesis. Targeting these pathways with therapeutics could be an effective preventative therapy