Oxidative stress: The achilles' heel of neurodegenerative diseases of the retina

Xue Cai[1], James F. McGinnis[1,2,3]

[1]Department of Ophthalmology, Dean McGee Eye Institute, [2]Department of Cell Biology, [3]Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

TABLE OF CONTENTS

1. ABSTRACT

Age-related macular degeneration (AMD) is the leading cause of blindness among adults in the developed countries. It is characterized by the progressive loss of central vision. AMD is classified into two forms: dry and wet. Dry AMD involves the accumulation of deposits in the RPE and Bruch's membrane; Wet AMD is characterized by neovascularization in the choroid. Whether the two forms of AMD share the same mechanism for the disease development is presently not clear. Oxidative stress, inflammation, and ER-stress are the common modes for the pathogenesis of AMD. In addition, other risk factors and several signaling pathways have been implicated as causative factors of AMD. In this paper, the mechanisms underlying AMD, risk factors involved in the pathology, representative animal models, and therapeutic treatment strategies are reviewed.

2. INTRODUCTION

AMD, a severe ocular disease, is among the most prevalent of ocular diseases. It occurs in adults at an age above 50 (1-2) with the loss of central vision and visual acuity. AMD development is progressive. By means of genetics, pathology, physiology, anatomy, biochemistry, animal models and functional research, a better understanding of the progression of AMD has been achieved. However, information about the mechanism underlying AMD is still insufficient, contributing to the absence of an effective treatment for AMD. This review paper is based on recent publications and identifies risk factors which contribute to the etiology as well as possible pathways involved in the disease development and therapeutic strategies for the treatment of AMD.

3. CHARACTERISTICS OF MACULAR DEGENERATION

Macular degeneration, an alternative name for age-related macular degeneration (AMD), is a heterogeneous human eye disorder that damages the center of the retina (called macula); is the leading cause of irreversible blindness among the adults over the age of 50 years; and is one of the major blinding diseases in the world (1-2). AMD is characterized by progressive loss of central, high-acuity vision due to photoreceptor degeneration in the central retina. AMD may exhibit the variable presence of soft and hard drusen in Bruch's membrane (BM) between the retinal pigment epithelium (RPE) and the choroid with or without evidence of damage to the underlying RPE, deposition of lipofuscin in the RPE, hyperpigmentation and hypopigmentation of the RPE. These features may occur in association with choroidal neovascularization (CNV), atrophy of RPE, geographic atrophy, neurosensory detachment, and fibrous scarring (3-4). The development of AMD is a progressive process and clinically it can be subdivided into early and late stages (2-3, 5). Early stage AMD exhibits normal visual acuity, but moderate vision loss associated with the focal lesion (drusen) or diffuse lesion (basal deposits) in BM in the fundus (2, 5). Late stage AMD involves CNV formation, in which blood vessels of the choriocapillaris grow into or through the BM; RPE detachment, which results in fluid accumulation between the RPE and BM; thickened BM with drusen; a high level of deposits of autofluorescence lipofuscin in RPE cells and loss of photoreceptors (2). Clinically, late stage AMD usually can be classified into two forms: "dry" and "wet" AMD (6). Dry AMD (geographic atrophy) counts for 80% of AMD cases and is characterized by focal degeneration of photoreceptors, RPE and choriocapillaris in the macula and impairs visual acuity over time, while wet AMD (neovascular) counts for 20% of the cases, and results in sudden and acute vision loss in patients due to choroidal neovessel development (6).

Currently it is not clear whether the disease processes and the genetic and molecular pathways that lead to the different phenotypes of both forms of AMD are related or distinct. Studies revealed that bone morphogenetic protein-4 (BMP4) is differentially expressed in dry and wet AMD. In dry AMD, BMP4 is highly expressed in RPE and mediates oxidative stress induced RPE senescence *in vitro* via Smad and P38 pathways whereas in wet AMD lesions, BMP4 expression in RPE is low. Evidence suggests that BMP4 is regulated by tumor necrosis factor (TNF) and may be the molecular switch determining which phenotypic pathway is taken in the progression of AMD (7-8). Furthermore, proteomic analysis of a total of 901 proteins from different developmental stages (early/mid stage or advanced stage) of dry or wet AMD and normal macular regions indicated that 56 proteins were elevated and 43 were reduced in AMD patient tissues compared to normal controls. About 60% of the elevated proteins are involved in immune responses and host defenses, including many complement proteins and damage-associated molecular pattern proteins.

These results indicate that different pathways are involved in the progression of the dry and wet AMD (9).

4. MECHANISM OF AMD

AMD has a complex and multi-factorial etiology. Genetic factors, oxidative stress, inflammation, age, caucasian race (ethnicity), high-fat diet, iron, color of the iris, obesity, hypertension, hypercholesterolemia, etc. are all contributors to the pathogenesis of AMD (10). Several pathogenic pathways have been proposed including oxidative stress-induced damage and apoptosis (11), RPE cell dysfunction with accumulation of lipofuscin and impairment of lysosomal functions (12) as well as inflammatory processes with complement activation (13).

4.1. Heredity effect (genetic variations)

The variation within the genome may occur at a single base pair (single nucleotide polymorphisms, SNP) or copy number variation of small blocks of sequences (14).

4.1.1. Complement system

The complement system is composed of more than 30 components and regulators. As an innate immune defense mechanism, the complement system has been suggested to play a significant role in the pathogenesis of AMD [see review (15)]. Complement activation involves three principal pathways which converge at C3 (complement component 3) activation and results in the generation of several effectors and ultimately the terminal MAC (membrane attack complex) which is a complex of C5b, C6, C7, C8, and C9, and which can directly cause lysis of the cells through the formation of lytic pores in the cell membrane. Several regulatory proteins, such as factor H (CFH), decay accelerating factor (CD55), membrane cofactor protein (CD46), and membrane inhibitor of reactive lysis (MIRLC or protectin (CD59), control the complement cascade and protect autologous cells from complement attack [see review (15)]. CFH is secreted by RPE and is present in drusen (16). It has been proposed as an inhibitor of C3 convertase and other complement pathways [see review (14-15)]. Its polymorphism, tyrosine-histidine substitution at amino acid 402 (Y402H polymorphism), could be a risk allele associated with AMD (17). FOXO3 (forkhead box, type O, member 3) belongs to the FOXO family of forkhead transcription factors which are key downstream targets of the phosphatidylinositol 3-kinase-Akt pathway. Analysis of the sequence and activity of mouse CFH promoter demonstrated that the FOXO3 acetylation mediated oxidative stress-induced CFH suppression, e.g. under oxidative stress conditions, FOXO3 preferentially binds to the CFH promoter, causing induction of CFH expression (18). On the other hand, selective knockdown of ocular CFH in a laser-induced mouse CNV model resulted in increased MAC deposition and lead to the early onset and exacerbation of CNV (19). However, other reports demonstrated that polymorphisms of the CFH gene which are in the noncoding regions (LOC 387715) are strongly associated with AMD and suggested it modulates the risk of AMD not by disrupting protein function but rather by regulating the CFH gene expression (20). Recently other complement-regulatory proteins such as

complement factors C2, C3, C5 and B have been reported to be risk factors for developing AMD (4, 15).

4.1.2. Other genes and DNA variations

Several other genes and DNA variations have been suggested to be associated with AMD. The DNA repair enzyme gene, XPD (xeroderma pigmentosum complementation group D) at codon 751 Gln/Gln genotype may have a protective effect against development of AMD as its presence is significantly lower in the dry AMD patients (21). Manganese superoxide dismutase (MnSOD) gene polymorphism (Ala-9Val sequence polymorphism) is much more frequently detected in both types of AMD patients (22).

Apolipoprotein E (ApoE) deficient mice at ages of 6, 12 and 20 weeks old showed a decrease of antioxidant enzymes, an increase of lipofuscin accumulation and pro-inflammatory cytokine expression (23). A combination of three established AMD risk factors, aging, high fat cholesterol-rich (HF-C) - diet, and APOE genotype, was demonstrated, using human apoE-targeted replacement (TR) or knockin mice, to be sufficient to induce AMD (5). In the aging apoE4 TR mice fed a HF-C diet, extensive degenerative alterations were seen at 65~127 weeks old of age in the retina/RPE/choroid. These changes are comparable to the pathology of human AMD including RPE hyperpigmentation, hypopigmentation, atrophy, BM thickening, soft drusenoid deposits, retinal neovescularization (RNV) and choroidal neovascularization (CNV) (5). Beta-amyloid (Abeta) was immunolocalized within the neovascularization (NV) and sub-RPE deposits in the aged apoE4 TR mice fed a HF-C diet, and within drusen and CNV in human AMD suggesting that apoE may promote amyloid deposition in AMD in an APOE4 allele-dependent manner (5).

4.2. Oxidative stress, inflammation and ER stress

Reactive oxygen species (ROS) include free radicals (O_2-·, OH·, HO_2·, ROO·), hydrogen peroxide (H_2O_2), or singlet oxygen ($^1O_2$). They may be produced as byproducts of cellular metabolism, or as byproducts of enzymatic reactions (24). Production of ROS and chronic oxidative stress lead to modification and damage of carbohydrates, membrane lipids, proteins and nucleic acids, which initiate the pathogenesis of many diseases [see review (25)]. The retina is the most frequently damaged organ following light exposure and has the highest rate of oxygen consumption than any other tissues in the body (24, 26). The outer segment (OS) membranes of the photoreceptor cells are rich in polyunsaturated fatty acids (PUFAs) (~50% of the lipid bilayer of the rod OS) which are particularly susceptible to free radical damage (24). The retina and RPE contain abundant photosensitizers (chromophores) including rhodopsin, opsin and 11-cis-retinal, that absorb light necessary for generating a chemical reaction for the visual cycle. In doing so, ROS continually "leak" from the active sites of the enzyme catalyzing these reactions (24, 26). Studies have demonstrated that phagocytosis by RPE eliminate up ~10% of rod and cone OS daily (27) and this process itself increases oxidative stress (24). Rohrer *et al* 2010 (28)

demonstrated that RPE cells treated with H_2O_2 reduce the levels of CD55 and CD59 to 70% and 60% of those untreated cells suggesting that oxidative stress resulted in a reduction of endogenous complement inhibitors and a increase in C3 binding and deposition. Totan *et al* 2009 reported that significantly higher malondialdehyde (MDA) (an index of lipid peroxidation), protein carbonyl (PC) (a marker of protein oxidation), 8-hydroxy-29-deoxygianosine (8-OHdG) (an indicator of oxidative DNA damage), total oxidation status (TOS) and lower total antioxidant capacity (TAC) levels were detected in the serum of wet AMD patients (29). Moreover, in the aqueous humor of wet AMD patients, the level of 8-OHdG was significantly higher and was positively correlated with the lesion size (30). Direct evidence that oxidative stress is a primary cause of AMD comes from the fact that deposits of lipofuscin (toxic, lipid-rich granules) in the RPE and drusen in the BM of AMD eyes contained oxidized proteins (glycoprotein) and lipids, complement components and amyloid (31-33). Conversely, treatment of AMD patients with antioxidants can slow the pathological progression and protect vision. Docosahexanoic acid (DHA) is the most oxidizable fatty acid and is highly concentrated in the photoreceptor OS and RPE cells (34-35). Its adducts are more abundant in the outer retina and serum of AMD patients (36). CEP (carboxyethylpyrrole), an oxidative fragment of DHA, had been identified in the drusen proteins (37). Immunization of C57BL/6J mice with mouse serum albumin adducted with CEP resulted in the development of antibodies to CEP, anchorage of C3 in BM, accumulation of drusen below the RPE during aging, a decrease in a- and b-wave amplitudes in response to light, and development of lesions in the RPE mimicking characteristics of dry AMD. Also, inflammatory cells are present in the region of lesions. This suggests that CEP, its precursor fragments, or CEP-adducted proteins are deposited in the BM and may be actively involved in initiating an inflammatory response as part of the pathology of AMD (38).

Chronic inflammation in the RPE cells has been implicated as a causative factor in AMD (39). Inflammatory cytokines IL-1 (interleukin-1), IFN (interferon), TNF, and various growth factors such as PDGF (platelet-derived growth factor) and FGF (fibroblast growth factor) have been shown to be involved in the pathogenesis of AMD (40). In Abcr$^{-/-}$ mice, A2E (N-retinylidene-N-retinylethanomalmine) accumulation causes enhanced secretion of VEGF (vascular endothelial growth factor), higher levels of expression of oxidative stress genes, complement activation, down regulation of complement-regulatory proteins and chronic inflammation of the RPE (41).

The endoplasmic reticulum (ER) is an important cellular organelle in which proteins are synthesized, folded, sorted and Ca^{2+} is stored. In recent years, more and more evidence implicating ER stress (protein folding stress), protein misfolding and aggregation as primary causes for many neurodegenerative diseases (42-44) has been accumulated. ER stress can be caused by many stimuli including oxidative stress, inflammation and other stress

conditions (42). It can be recognized by three transmembrane proteins called IRE1 (inositol-requiring protein-1), PERK (protein kinase RNA-like ER kinase) and ATF6 (activating transcription factor-6) which act as ER-resident sensors. In response to ER stress the cells generate a self-protective UPR (unfolded protein response) signaling cascade through protein kinases and transcription factors by regulation of ER chaperons, antioxidants and other guardian molecules (42). Under ER stress, the UPR signaling pathways are initiated for the protein proper folding and protein degradation to prevent cellular damage. If the ER stress is prolonged, apoptosis will eventually be initiated by regulation of caspases and CHOP (CCAAT/enhancer-binding protein homologous protein) expression (42-44). Proteomic analysis of retinas from human AMD patients demonstrated that chaperon proteins HSP60 (heat shock protein) and HOP (Hsp70/Hsp90 organizing protein) which are responsible for protein folding, were down regulated and a group of proteins involved in microtubule formation and regulation were also decreased in expression levels (45). The precursor to ER resident chaperon protein ERp29, which is associated with neurodegenerative diseases, was significantly lower in the eye of Ccr2$^{-/-}$/Cx3cr1$^{-/-}$ [chemokine CCL receptor (CCR2) and chemokine C-X3-C receptor 1(CX3CR1) double knockout] mice (46). These down regulated proteins may result in the blockage of protein trafficking, and subsequent accumulation of unfolded protein aggregates and deposition of drusen and lipofuscin which eventually cause damage to RPE and BM.

Many other genes and factors are shown to be involved in oxidative stress. Tissue factor (TF), the primary initiator of blood coagulation initiates intracellular signaling and promotes inflammation and angiogenesis. TF was shown to be up regulated in the retinas of AMD patients and Ccl2$^{-/-}$/Cx3cr1$^{-/-}$ double knockout mice. LPS (lipopolysaccharide) and H$_2$O$_2$ treatment of ARPE-19 cells increased TF expression (47). PPARs (peroxisome proliferator-activated receptors) belong to the steroid/thyroid nuclear receptor superfamily of ligand-activated transcription factors and play a role in AMD progression by regulating VEGF, MMPs (matrix metalloproteases), DHA, heme-oxygenase-1 (HO-1) and ROS (48). PPAR gamma, one of the three subtypes of PPARs, has been reported to be expressed in ocular tissue, specifically in RPE cells (49). In Ccl2$^{-/-}$/Cx3cr1$^{-/-}$ mice, AMD patients and H$_2$O$_2$ treated ARPE-19 cells, the expression of PPAR gamma and its downstream proteins, VEGF, MMP-9 and HO-1 was increased (49). IL-6 (interleukin-6), a key factor in the modulation of immune responses and inflammatory processes was increased in AMD whereas its production was stimulated by H$_2$O$_2$ in ARPE-19 cells (50).

Knockdown of superoxide dismutase 2 (SOD2) in mitochondria by ribozyme (AAV-Rz432) in C57BL/6J mice resulted in decreased levels of MnSOD expression, elevated levels of markers of oxidative damage (nitrated and carboxyethylpyrrole-modified proteins) in the RPE-choroid. The mice exhibit multiple phenotypes similar to human AMD such as thickened BM, degeneration of RPE, shortened and disorganized photoreceptor outer and inner segments, apoptotic cell death, increased autofluorescence and elevated levels of A2E and iso-A2E (51).

Oxysterols are oxidation products of cholesterol that result from either autoxidation or enzymatic oxidation. Abnormal oxysterol levels can cause oxidative stress, inflammation and apoptotic cell death (52). The oxysterol pathway has also been proposed as a unifying hypothesis for the cause of AMD (52). APRE-19 cells treated with cholesterol oxidation metabolite 27-hydroxycholesterol (27-HOC) for 24 hours increased levels of Abeta peptide production, ER stress markers, and oxidative stress-activated protein nucleus factor-kappaB (NF-kB) and HO-1. Furthermore, 27-HOC causes depletion of ER Ca^{2+} stores and glutathione, ROS generation, inflammation and apoptosis-mediated cell death (53).

Ubiquitin proteolytic system (UPS), distributed in different retinal cell types, had been demonstrated in the protection of retina from oxidative stress by mediating the degradation of misfolded and oxidative-damaged proteins without selection through modulation of the stability and activity of transcription factor Nrf2 (nuclear factor erythroid-derived 2, like 2) (54-55). However, the UPS only degraded the newly synthesized proteins (55-56). The accumulation of protein aggregates in AMD patients are newly-synthesized oxidative-damaged proteins. So it is possible that enhancing the capacity of UPS to degrade these proteins may provide a new strategy for treatment of AMD.

Several signaling pathways have been proposed to correlate with AMD including VEGF-A/VEGF-R2/PI3K/Akt (57), PERK/eIF2a/ATF4 and IRE1/ASK1/JNK cascade (42). A missense mutation in fibulin-3 protein (R345W) caused protein accumulation in the ER of RPE cells which triggered the activation of UPR and lead to the increase of VEGF expression. This evidence suggests a possible link between ER stress and wet AMD (58).

4.3. Cigarette smoking

Cigarette smoke is the most important environmental risk factor contributing to both dry and wet AMD pathogenesis (59) by causing oxidative damage and RPE cell death (60). ARPE-19 cells and primary human RPE cells exposed to cigarette smoke extract or a component of cigarette smoke (hydroquinone, HQ) showed oxidative damage, reduced cell viability and apoptosis including reduction in cell size and nuclear condensation, increased lipid peroxidation (determined by increased synthesis of 4-hydroxy-2-nonenal, 4-HNE) and mitochondrial superoxide production, and decreased intracellular glutathione (GSH) which is an important antioxidant that aids in eliminating toxic chemicals (61). However, cigarette smoke also induced the expression of VEGF, HO-1 and the transcription factor Nrf2 (61). Two month old C57BL/6J mice exposed to smoke for 5-hour/day, 5-day/week for 6 months produced oxidative stress-associated features compared to control mice, including an increase in 8-oxo-7,8-dihydro-2'-

deoxyguanosine (8-OHdG), a higher percentage of apoptotic RPE, thicker BM and ultrastructural evidence of oxidative damage in the RPE and BM, such as increase in intracellular vacuoles, higher basal laminar deposits and outer collagenous layer deposits (62). Low levels of MCP (monocyte chemoattractant protein) -1 protein were detected in RPE from AMD smoker patients and in ARPE-19 cells and RPE/choroid from C57BL/6J mice of HQ-induced oxidative injury. VEGF protein was increased and PEDF (pigment epithelium-derived factor) protein was decreased in RPE from AMD smoker patients and smoking treated mice (63). Furthermore, heat shock protein 27 (Hsp27), a key regulator of actin filament dynamics, is up-regulated in RPE from patients with AMD, and Hsp25, p38, and ERK (extracellular signal-regulated kinase) phosphorylation are increased in aging C57BL/6J mice which were chronically exposed to HQ, suggesting that phosphorylated Hsp27 might be a key mediator in the pathogenesis of AMD along with actin reorganization and bleb formation (64).

4.4. Other factors for AMD

AMD patient retinas contained more iron than the healthy subjects which suggests that excessive iron can cause damage to protein, lipids and DNA through the generation of free radicals in the Fenton reaction (65). Ceruoplasmin (Cp) and hephaestin (Heph) are multicopper ferroxidases and can facilitate iron export from the cells. Iron can be taken up by the serum transport protein transferrin (66). In a Cp and Heph double knockout mouse model which exhibits both wet and dry AMD characteristics, iron overload was found in the retina in an age-dependent manner with a reduced level of transferrin receptor (66).

Recently, Lyzogubov *et al* 2011 subretinally injected 1 mg polyethylene glycol-8 (PEG-8) to wild type mice and caused elevated levels of C3 split products, C9, TGF (transforming growth factor) –beta 2, bFGF (basic fibroblast growth factor) at post injection day 1, and the progression of developed CNV to penetrate into the BM at day 3, fully developed CNV and retinal degeneration occurred at post injection day 5 (67).

5. ANIMAL MODELS OF AMD

Mice have been widely used to generate models for investigation of the pathogenesis of human AMD. Because of the complication in the pathology of AMD, there is no good mouse model displaying all the phenotypes of AMD. For example, the mouse has no macula, a lower ratio of cone to rod, drusen is rarely seen due to their simpler BM and different lipofuscin extrusion compared with humans. However, focal atrophy of photoreceptor and RPE, lipofuscin accumulation, and increased A2E levels can develop in aged mouse eyes, which are representative of the distinct characteristics of AMD (68). AMD models can be generally divided into three groups (68). In the first group are genetically engineered mice that target genes related to juvenile macular dystrophies including Abcr knockout (recessive Stargardt disease) and transgenic ELOVL4 (dominant Stargardt disease) or target

inflammatory genes relevant to AMD such as Ccl2, Cfh$^{-/-}$, Cx3cr1$^{-/-}$, and Ccl2$^{-/-}$/Cx3cr1$^{-/-}$. Also, oxidative stress associated genes such as Sod1 and Sod2 knockdown and metabolic pathway genes such as transgenic mcd/mcd (cathepsin D), and transgenic ApoE4 on high fat and high cholesterol diet (lipid metabolism) are in this group. The second group consists of natural mouse strains such as arrd2/arrd2 (Mdm gene mutation) and the senescence accelerated mice (SAM) which spontaneously develop features of dry AMD (68). The third group of mice was immunologically or mechanically manipulated by immunization with CEP (an oxidative fragment of DHA found in drusen), injection of vectors containing VEGF or peptides, or laser photocoagulation (3-4, 68). We have selected several representative mouse models for dry, wet and both forms for AMD to discuss the phenotype and disease mechanisms of AMD.

5.1. Dry AMD models

ABCR: ABCR, a retina-specific ATP-binding cassette transporter sub-family A member 4 (ABCA4), is a multiple-span transmembrane protein which is exclusively localized in the rim region of OS discs (69-70). It acts as a flippase for removing all-trans-retinal and its derivatives from the OS disc lumen to the cytoplasm for the reduction of all-trans-retinol for the visual cycle by using energy via ATP hydrolysis (71-73). Mutations in the ABCR gene cause a wide spectrum of retinal degenerative diseases including Stargardt macular degeneration (74) and AMD (75). The Abcr knockout mouse (Abcr$^{-/-}$) was created by replacing 4.0 kb of the promoter region plus exon 1 by a PGK-neo cassette (71). Abcr$^{-/-}$ mice show delayed rod dark adaptation and recovery following light exposure with abnormal clearance of all-trans-retinal, which results in the accumulation of N-retinylidene phosphatidylethanolamine (N-ret-PE) and all-trans-retinal in the OSs (71-72). High levels of lipofuscin in the RPE cells which exhibit as electron density bodies under electron microscopy and elevated levels of lipofuscin fluorophores, A2PE-H2 and A2E, are also seen in the RPE cells (71-72). Furthermore, higher levels of expression of oxidative stress genes and elevated lipid peroxidation, as well as elevated levels of complement-activation products and down regulation of complement-regulatory proteins (CRPs) in the Abcr$^{-/-}$ eyes were observed (41). These data suggested that A2E accumulation causes oxidative stress, complement activation and reduced CRPs levels, all of which induced chronic inflammation of the RPE (41). The progression of photoreceptor degeneration is very slow in the Abcr$^{-/-}$ mice (76). In albino background, Abcr$^{-/-}$ began photoreceptor cell loss at 8 months of age and worsened after that (77). Therefore the AMD phenotypic is due to the atrophy of RPE rather than photoreceptor degeneration in this mouse model.

ELOVL4: ELOVL4, an ER resident membrane-bound protein in the retina, belongs to the ELO (elongation of long chain fatty acid) family and participates in the biosynthesis of very long-chain fatty acids and docosahexaenoic acid (DHA) (78-79). Recent studies demonstrated that ELOVL4 is required for the synthesis of C28 and C30 very long chain saturated fatty acids (VLC-

FA) and of C28-C38 very long chain polyunsaturated fatty acids (VLC-PUFA) (80). Mutation of this gene results in loss of its retention in the ER with subsequent mislocalization as aggregates suggesting that the mutant protein is misfolded/unfolded and results in the UPR activation (81). The mutant protein is also unable to target to the ER for fatty acid biosynthesis (80) thus causing Stargardt-like macular dystrophy (STGD3) and atrophic macular degeneration (dry AMD). The transgenic mouse was created by introducing a 5-bp deletion of nucleotides (AACTT) at 790 –794 bp of the human wild type ELOVL4 gene (82). In the Elovl4$^{-/-}$ mice, high A2E levels were detected in the RPE cells at 2 months of age even in lines that express very low amounts of transgenic protein, and lipofuscin accumulates in the RPE at 7 months of age (83). Failure to form normal OS in the photoreceptors was seen in lines expressing higher levels of the transgene. The loss of 50% of photoreceptors occurs at 6 weeks, 16 weeks and 18 months in higher, middle and lower ELOVL4-expressing lines, respectively, correlated with decline in retinal function (82).

5.2. Wet AMD models

CNV: CNV models are commonly used for neovascularization development and therapeutic treatment for wet AMD. These animal models are induced by laser photocoagulation, surgical and genetically engineered (84). Laser-induced CNV disrupts BM and results in a high frequency (100%) of the development of CNV within 2~3 weeks (85). This model has the advantage that it is inexpensive, reproducible, relatively easy to create and the rapid onset of the CNV ensures the experiment can be processed and finished in a relatively short time period. However, the variable rate of CNV and transient CNV leakage are the major disadvantages of this animal model (84).

Vldlr: Transgenic Vldlr$^{-/-}$ mouse, created by a partial deletion of exon 5 in the very low density lipoprotein receptor (Vldlr) gene on chromosome 19, is characterized by progressive and developmental neovascularization (86) and is a model for RAP (retinal angiomatous proliferation) in humans (87). In contrast to typical wet AMD in which the new blood vessels arise from the choroid extending to the subretinal space, the pathologic new blood vessels in RAP originate from the inner retina (outer plexiform layer) at postnatal day (P) 13~14, extend through the photoreceptors, invade the subretinal space and choroid, and eventually cause RPE disruption, BM exposure and photoreceptor degeneration with significant fibrosis (86-88). The development of abnormal leaky new vessels reaches the maximum at P42 (86-87). Decreased mRNA levels of rhodopsin and cone-opsin were detected around 2 months of age but retinal degeneration and ERG amplitude reduction occur late, at 3 months (89) and 6 months of age (90), respectively. Decreased levels of 11-cis-retinal and retinyl esters occurred at an old age (8 months) (89). Vldlr$^{-/-}$ mice have severe retinal vascular leakage and impaired blood-retinal barrier (89), and exhibit up regulation of pro-inflammatory factors, adherent leukocytes and molecules as well as increased NF-kB expression (89, 91). In addition, significant higher levels of VEGF and bFGF, and GFAP (glial fibrillary acidic protein) were shown in the Vldlr$^{-/-}$ mice (91-92). These observations suggest that up-regulation of

inflammatory response factors and activation of MAPK/Akt/NF-kB signaling cascade, activation of Müller cells, along with elevated VEGF and bFGF expression, all contribute to neovascularization in the Vldlr$^{-/-}$ mice (91). Furthermore, elevated LRP5/6 (low-density lipoprotein receptor-related protein 5 or 6) and free beta-catenin expression levels indicated that wnt (wg/int1, wingless/integration 1) signaling pathway was activated in Vldlr$^{-/-}$ mice and knockdown of the expression of Vldlr by siRNA proves that Vldlr is a negative regulator of the wnt signal pathway and a positive regulator of angiogenesis (92). Dickkopf-1 (wnt pathway inhibitor which binds LRP5/6) treatment increases phosphorylated beta-catenin, and decreases VEGF and free beta-catenin levels demonstrating that Vldlr-deficiency causes VEGF overexpression and CNV through the wnt signaling pathway (92).

5.3. Both wet and dry AMD models

SOD1: Sod 1 (superoxide dismutase 1) deficient (Sod1$^{-/-}$) mice exhibit many features seen in wet AMD patients (93-94) such as progressive photoreceptor degeneration with drusen, thickened BM, and choroidal neovascularization in the older mice (94-95). ONL (outer nuclear layer) thickness at 10 weeks is not different from the WT (wild type); significant loss of ONL starts at 30 week and 50 weeks in INL (inner nuclear layer); the ultrastructure of the retina at 15 months old mice showed oxidative stress associated appearance including swollen nuclei, vacuolized cytoplasm, disrupted membranes and damaged mitochondria. ERG assessment of the retinal function before 40 weeks showed no difference from the WT, and both the rod and cone function showed no reduction at 40 weeks of age (95). The mice with the G86R mutation of SOD 1 gene exhibited a diminution of electroretinographic activity following exposure to constant bright light for 20 days and specific degeneration of photoreceptor cells (96).

Ccl2/Cx3cr1: The Ccl2$^{-/-}$/Cx3cr1$^{-/-}$ double knockout transgenic mouse, a model characterized with lower fertility, develops early onset, at 6 weeks of age, of retinal lesions similar to human drusen. Other prominent features include abnormal RPE cells, thickened BM with drusen which are smaller than drusen in AMD patients, photoreceptor degeneration and significantly high levels of A2E accumulation, choroidal neovascularization and decreased levels of ER resident chaperone protein, ERp29, in the retina (46). When Ccl2$^{-/-}$/Cx3cr1$^{-/-}$ mice were fed with either a high or low omega-3 LCPUFA diet, retinal lesions were observed at 6 weeks of age in both groups but the number of lesions was decreased in the mice that ingested a high omega-3 LCPUFA diet compared to control group which developed RPE changes, progressive neovascularizations and drusen-like lesions as revealed by fundoscopy, histology and ultrastructure (46).

6. THERAPIES OF AMD

Because the pathogenesis of AMD is complicated and largely unknown, there is no effective and promising single treatment for prevention and/or cure. Destruction, or slowing the formation, of CNV by laser photocoagulation or anti-VEGF therapy have been shown to be the most successful treatment, but no proven therapy is available for RPE detachment, geographic atrophy, and early stage

AMD (2). Currently, ranibizumab (lucentis) and pegaptanib are two anti-VEGF agents approved by the US Food and Drug Administration (FDA) for treatment of neovascular AMD and they significantly improve the visual acuity of wet AMD patients. Bevacizumab (avastin), a commonly used anti-VEGF agent similar to ranibizumab, was shown to be effective for two years and was well tolerated and safe in most patients [see review (97-99)]. However, the treatment requires repeated dosing about once a month because neovascularization occurs through multiple signaling pathways (100) and frequent intravitreal injection could cause eye damage (2, 101). Multiple therapeutic strategies which address effectiveness, safety, cost, dosing and efficacy are being development including gene therapy, anti-angiogenic factors, and blockage of signaling pathways (102).

6.1. Pharmacological treatment
6.1.1. Antioxidant treatment
N-acetyl-cysteine, a precursor of glutathione and a potent antioxidant, was intraperitoneally injected into laser photocoagulation-induced CNV mice and 4-HNE-modified protein and activation of NF-kB in nuclear extract were markedly suppressed after 3 hours and 6 hours respectively. Also, macrophage and neutrophil recruitment were inhibited, the levels of MCP-1, chemokine (C-X-C motif) ligand (CXL)-1, VEGF, and VEGF receptor (VEGFR) -1 were reduced, and the extent of CNV was significantly reduced by7 days after treatment (103).

Lutein (LUT) and zeaxanthin (ZEA) are plant carotenoids that are present in a normal diet and major components in macular pigment (104-105). Increased dietary intake of LUT and ZEA resulted in increased plasma levels which were positively and significantly associated with macular pigment optical density (104). Pretreatment of rat retinal neurons in culture with DHA, LUT, ZEA or BC (beta-carotene) alone, or LUT, ZEA with DHA reduced oxidative stress-induced apoptosis in photoreceptors, preserved the mitochondrial membrane potential, cytochrome C translocation from mitochondria and enhanced photoreceptor development, survival and differentiation (105).

$Sod1^{-/-}$ mice treated with a mixture of antioxidants experienced a significant reduction in RNV, and wild type mice treated with the same drug also reduced RNV and CNV (93).

Zinc supplementation has prevented blindness in 25% of the patients with dry AMD by effectively decreasing oxidative stress and inflammatory cytokines (106).

Omega-3 fatty acids and their acid metabolites, resolvins and protectins, are endogenous anti-inflammatory compounds which can prevent NF-kB signaling, thus providing a putative target for AMD treatment (107). $Ccl2^{-/-}$/$Cx3cr1^{-/-}$ mice were fed with either a high or low dose of long-chain omega-3 polyunsaturated (n-3) fatty acids (omega-3 LCPUFA). Although retinal lesions were observed at 6 weeks of age in both groups, the number of lesions was decreased in the mice that ingested a high omega-3 LCPUFA diet compared with the low n-3 fatty acids group (46, 108). And in the control group deprived of the omega-3 LCPUFA, the retinal lesion continued to progress (46).

α-tocopherol is a vitamin E component and is a major lipid-soluble chain-breaking antioxidant in mammals. Acrolein, a product of lipid peroxidation caused significant loss of ARPE-19 cell viability and a series of oxidative–associated reactions. Pretreatment of ARPE-19 cell with α-tocopherol activated the Keap1/Nrf2 pathway which increased the expression and/or activation of several phase II enzymes, consequently improving mitochondrial function, lowered ROS and protein oxidation levels and protected RPE cells from acrolein-induced damage while enhancing cell viability (109).

Melatonin has receptors localized in RPE cells and has the potential to prevent telomere shortening. Clinical trials showed that orally taking 3 mg melatonin each night for 3 months reduced pathologic macular changes in AMD patients (110).

Treating Abcr-/- mice with isotreatinoin resulted in complete blockage of new synthesis of A2PE-H2, A2E and A2E-oxiranes and reduced lipofuscin accumulation (111). Vitamin A and fenrerinide (an inhibitor of vitamin A) can significantly increase the levels of retinyl esters and reduce lipofuscin accumulation in the $Abcr^{-/-}$ mice (112).

6.1.2. Antiangiogenic treatment
Carboxyamidotriazole (CAI), an anti-angiogenic and anti-tumor agent, was formulated with aqueous beta-hydroxypropyl cyclodextrin (bHPCD-CAI) and intravitreally administered to a laser-induced CNV mouse model. Its effects on reduction of CNV lesion volume occurred in a dose-dependent manner. Pharmacokinetics demonstrated that there is a high concentration of CAI in the vitreous compartment without ocular toxicology (113). Antiangiogenic drugs such as lodamin, CAI, or nerve growth factor receptor inhibitor K252a significantly regressed or inhibited laser induced CNV in mice (113-114).

Immune response depends on the constitutive expression of FasL, a death-inducing ligand, the expression of which inhibits inflammation and tumor growth by inducing apoptosis in cells (115). FasL expression in the RPE cells plays an important role in controlling neovascular diseases (115). Laser-induced CNV mice were injected intravitreally with recombinant soluble human FasL, or treated with doxycycline (an MMP inhibitor) in the drinking water. Both antiangiogenic agents resulted in prevention of neovascularization on day 7 (FasL) or day 1 and 3 (doxycycline) (116).

6.1.3. Various factor inhibitors
Insulin-like growth factor-1 is involved in ocular neovascularization. Its receptor (IGF-1R) inhibitor PPP (cyclolignan picropodophyllin) reduced the CNV area and

significantly decreased the VEGF levels in the choroid in laser-induced CNV mice (117).

Weekly intravitreal injections of compstatin (50 micro gram), a complement component 3 (C3) inhibitor, into 16-year old and 4-year old monkeys with dry AMD resulted in a diffusion of drusen in the macular region at 6 months after injection whereas monthly injections of 1 mg of compstatin resulted in the partial disappearance of drusen was observed by 9 months after injection (118).

Notch signaling occurs via a mechanism which plays a myriad of roles during vascular development and recent studies suggest that its regulation of angiogenesis is in concert with the VEGF pathway (119-121). VEGF receptor (VEGFR-2) is an important receptor tyrosine kinase which plays a premier role in the normal vascular development and neovascularization (122). Inhibition of Notch signaling (121) or VEGFR-2 expression by peptide vaccination (antigen peptide of human VEGFR-2) (123) reduced the CNV lesion volume to 18% and retinal vasculature leakage to 80%. Also, orally administered VEGFR-2 inhibitors significantly decreased the fluorescein leakage and the volume of CNV membrane (124-125).

6.2. Gene therapy
Overexpression of glutathione peroxidase 4 (Gpx4) *in vivo* by transgenic mice with inducible expression of Gpx4 in photoreceptors strongly protected retinal structure and function in oxidative damage-induced retinal degeneration. It also reduced oxidative stress induced RPE cell damage. Furthermore, overexpression of Gpx4 provided a better therapeutic benefit than overexpression of SOD1 or SOD2 although they are important endogenous defense systems (126).

Subretinal delivery of C3 expressing recombinant adenovirus (adcmvC3) to adult murine RPE cells induced significant morphological changes which resemble features of AMD, such as significantly increased vascular permeability, endothelial cell proliferation and migration, and RPE atrophy (127).

RPE deterioration and CNV development require the activation of complement alternative pathway (CAP). Abnormalities of CAP regulation lead to the inflammation, drusen formation and the development of AMD. Complement factor H (CFH) is a CAP control protein. A recombinant form of CFH, CR2-CFH, containing the N-terminal domain of mouse CFH encoding CAP-inhibitor and linked to a complement receptor 2 (CR2) encoding a target fragment for C3 component binding, was demonstrated to reduce the loss of RPE integrity and decrease angiogenesis in CNV mice. Also, the expression of VEGF and C3 was significantly reduced and retinal function was restored to about 80% of that in the wild type mice (28).

Selective knockdown of ocular CFH in a laser-induced mouse CNV model resulted in increased MAC deposition and lead to the early onset and exacerbation of CNV (19). CD59 is a complement regulatory protein controlling the formation and function of MAC when it presents on the cell surface. It can bind to C8, C9, or both in the MAC complex and prevents the polymerization of C9 and the formation of MAC (128). CD59, with a membrane targeting moiety (APT542), can firmly bind to the cell surface. Treatment of laser-induced CNV mice with recombinant, soluble membrane-targeted CD59 (rCD59-APT542) (129) blocked the complement activation and the formation of MAC, reduced the size of fully developed CNV by 79%, increased apoptosis and decreased cell proliferation in the neovascular complex. Overexpression of human CD59 (hCD59) by delivering an adenovirus (Ad) vector into murine RPE cells (130) prevented MAC deposition and MAC-induced damage to RPE cells.

CCR3 (eosinophil/mast cell chemokine receptor) and its ligands eotaxin-1, -2, and -3 are specifically expressed in choroidal neovascular endothelial cells in humans with AMD. Blockade of CCR3 (anti-CCR3 neutralizing antibodies) or its ligands resulted in the suppression of CNV in a laser-induced CNV mouse model. Data demonstrated that this is due to direct inhibition of choroidal endothelial cell proliferation and is uncoupled from inflammation and independent of macrophage and neutrophil recruitment. Furthermore, it proved to be more effective than anti-VEGF-A treatment and is not toxic to the retina (131). However, immunostaining showed no specific expression of CCR3 in or near CNV indicating that CCR3 does not play a direct role in CNV development and questions the therapeutic approach of targeting CCR3 to suppress CNV (132).

AAV-mediated expression of a soluble form of VEGF receptor (Flt) 1 (AAV.sFlt 1) which only contained the extracellular domains of the Flt-1 protein, was subretinally delivered to trVEGF029 mice (133) or laser-induced CNV mice and monkeys (134). This lead to sustained sFlt expression for 8 months (mice) and 17 months (monkeys) and was accompanied by 8 months regression of CNV (134). The retinal neovascularization in the ONL was reversible; photoreceptor numbers and retinal function were retained (133-134). Similarly, AAV5.sFLT01, in which the Flt-1 signal peptide sequence was directly fused to Flt-1 domain 2 and linked to a human IgG1-Fc region was subretinally injected into Ccl2^{-/-}/Cx3cr1^{-/-} mice. This resulted in arrested retinal lesions, lowed A2E levels, reduced lipofuscin accumulation and decreased levels of ERK signals 3 months after treatment (135).

6.3. Regenerative medicine
During the past several years, stem cell-based therapeutic applications in retinal degenerative diseases have attracted more attention because of their potential for advanced treatment of diseases (136-137). Stem cells are a group of pluripotent cells which can enter the circulatory system and, when needed, differentiate into specialized cells (137) to generate various organs. Currently, many published reports indicate that stem cells from a variety of cell types can differentiate into photoreceptor cells or RPE cells as indicated by expression of key markers of these

ocular cells [see review (137)]. Compared to gene therapy, which is effective for the treatment of early stage diseases, the stem cells have the potential to treat advanced ocular diseases (136). The Ali research group reported in 2006, the transplantation of neural retinal cell suspensions from GFP (green florescent protein) transgenic (Nrl-GFP$^{+/+}$) mice at postnatal day 1, into the subretinal space of GFP-negative wild type littermates and adult recipients. The mixture of proliferating progenitors, post-mitotic precursors and differentiated cells that do not yet express the markers of mature photoreceptors, migrated into the retina and displayed the morphological features of mature photoreceptors three weeks later (138). Furthermore, they revealed that P3~5 donor cells from retina showed the greatest integration, but donor cells from other ages can only survive without integration. Three mouse models of inherited retinal degeneration: adult retinal degeneration slow (rds), P1 retinal degeneration fast (rd) and a 4 week rhodopsin knockout (rho$^{-/-}$) were tested by subretinal injection. P1 Nrl-GFP$^{+/+}$ donor cells integrated into the retina of adult rds mice and remained viable for at least 10 weeks. Peripherin-2, which was absent in the adult photoreceptors, was seen in the short outer segments emerging from transplanted cells. Nrl-GFP$^{+/+}$ donor cells can survive in the rd retina for 3 weeks, an age which the ONL of the rd mouse retina has been reduced to a single layer. Transplanted Nrl-GFP$^{+/+}$ cells integrated into the recipient ONL of rho$^{-/-}$ mice, and rhodopsin was immunolocalized to the outer segments (138). Moreover, the transplant cells were more sensitive to the light-evoked responses at relatively lower light intensities than the rho$^{-/-}$ sham injected eyes (138).

Stem cells have been isolated from adult tissues and shown to have the capacity of self-renewal and regeneration for ocular structures, thus providing many potential treatments for wet AMD (139-140). Human embryonic stem cell-derived RPE (hES-RPE) differentiate into functionally polarized hES-RPE cells as early as 4 weeks and had a normal chromosomal karyotype. Phenotypically polarized hES-RPE cells showed expression of RPE-specific genes, prominent expression of PEDF which was secreted into the medium and enhanced retinal progenitor cells (RPCs) survival (141). Adult bone-marrow-derived endothelial progenitor cells (EPCs) were reported to be capable of incorporating into the existing blood vessels after injection into the vitreous of new born mice and forming vascular mosaics and endogenous retinal vascular endothelial cells (142). Patients with AMD and mice with laser-induced CNV expressed high levels of collagenase-3 (MMP13), a matrix metalloproteinase (MMP) family member. A deficiency of MMP13 in mice was shown to inhibit the formation of laser-induced CNV suggesting that MMP13 is required for CNV. Several injections of mesenchymal stem cells (MSCs) from wild type bone marrow fully restored CNV indicating that these stem cells also participated in CNV (143). Engineered bone marrow-derived MSCs were intravenously injected into a laser-induced CNV mouse model and were specifically recruited into CNV lesions where they differentiated into multiple cell types and participated in the neovascular development. Furthermore they produced

PEDF to inhibit the growth of CNVs and regression of CNV which were mediated by RPE cells (144). Bone marrow cells from enhanced GFP (EGFP) transgenic mice were transplanted into the wild type adult, three months later CNV was induced by photocoagulation, the vascular wall cells of the CNV expressed EGFP and CD31. This result demonstrated that bone-marrow-derived stem cells participated in the development of the neo-blood vessels in the CNV (145).

6.4. Nanomedicine

Nanoparticles, because of their tiny size and large surface area, have proven to be ideal carriers for effectively and efficiently delivering drugs, nucleotides and peptides to the eye (146-147). Some metal nanoparticles have shown themselves to be direct antioxidants and are able to block increases of ROS (148). Rare earth cerium oxide nanoparticles (nanoceria) have a high capacity to reverse oxygenation/deoxygenation without alteration of the fluorite lattice structure (149). They can exist as, and switch between, the +3 (fully reduced) and +4 (fully oxidized) valence states in a redox reaction. This results in their ability to scavenge free-radicals due to an increase in oxygen vacancies in the surface of the crystal structure and by the loss of oxygen and/or its electrons (149-150). The unique characteristics and capabilities of nanoceria, such as biocompatibility, small size, and cell/nuclear membrane permeability, have proven them to be excellent agents for biological applications (151-153). Nanoceria exhibit catalytic activities of the two antioxidative enzymes superoxide dismutase and catalase (154), and they act as direct antioxidants to inhibit ROS-induced death of a variety of cells *in vitro* and *in vivo* (151, 154-159). Nanoceria have no genotoxicity, are not toxic to cultured cells and are non pathologic to mouse tissues (154, 156-159). Due to their unique regenerative properties, nanoceria do not require repeat dosages as seen with the use of dietary supplements of other antioxidants.

Our lab has shown that nanoceria can be stored in a phosphate buffered saline at room temperature for a couple of years and still maintain their stability and effectiveness (unpublished data). Almost 80% of nanoceria injected intravitreally are retained in the albino rat retina for at least 4 months as determined by inductively coupled plasma-mass spectrometry (ICP-MS) (159). In addition, nanoceria have been shown to prevent photoreceptor death and to rescue retinal function in light-damaged rats (151, 155). They also slow photoreceptor degeneration and improve retinal function in an inherited retinal degenerative mouse model (tubby). This mouse has a splicing mutation in the Tub gene and is a phenotypic model of Usher syndrome in humans. The nanoceria protection occurs by up regulating survival signaling pathways and/or down regulating apoptosis signaling pathways (160). In addition, nanoceria were successfully employed to inhibit developing neovascularization in the Vldlr$^{-/-}$ mice when intravitreally administered before the disease onset (P7) (161). Their effect on down regulation of VEGF levels is concentration-dependent with a total dose of 172 nanograms in 1 micro liter providing the highest benefit (unpublished data). By preventing increases in ROS, nanoceria inhibit VEGF
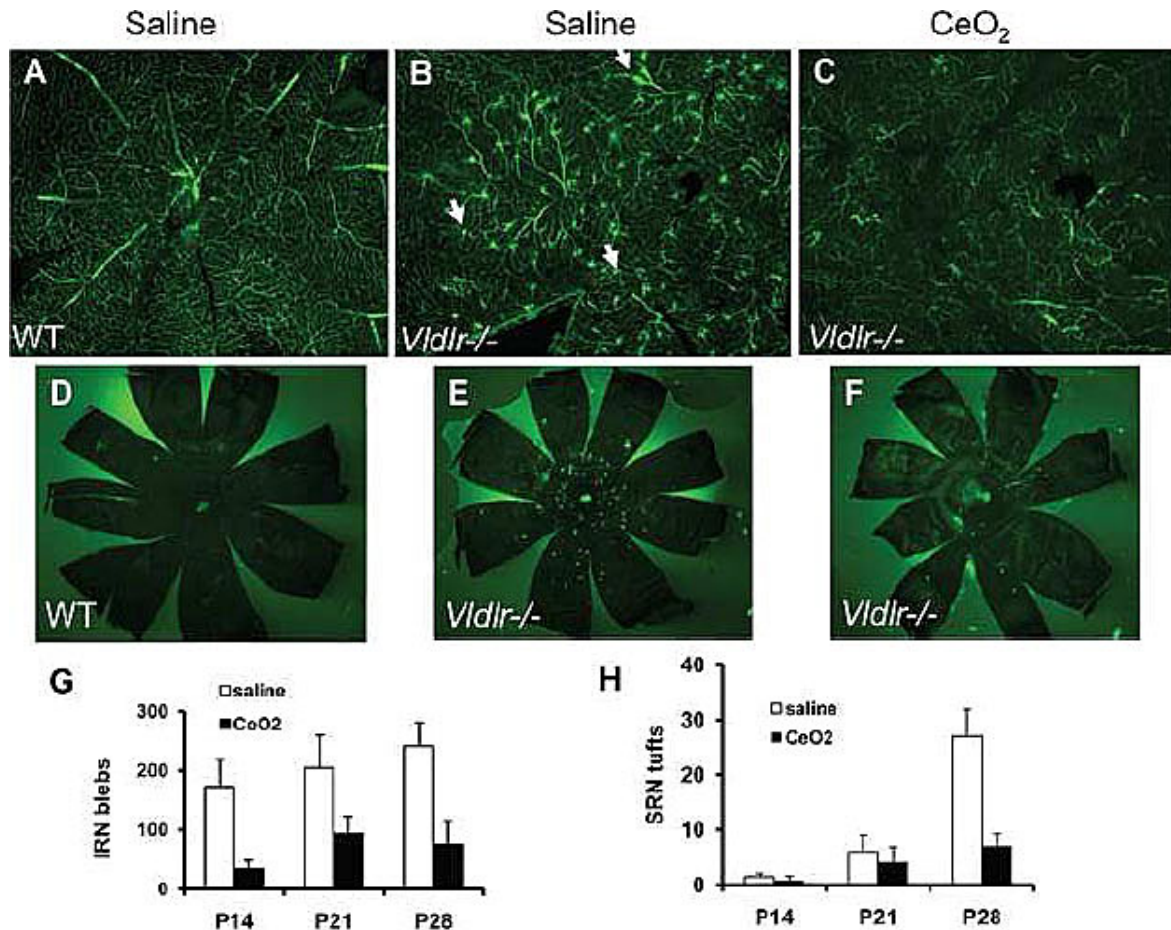
Figure 1. Nanoceria prevent abnormal development of pathologic blood vessels in the retina (top row) and choroid (bottom row) at P28 after single injection at P7. All retinal blood vessels were labeled green by the vascular filling assay. Wild type (WT) retina shows web-like vasculature (A) and choroid shows no choroidal "tufts" (D) whereas Vldlr^{-/-} retina exhibits numerous intraretinal vascular "blebs" (arrows) (B) and Vldlr^{-/-} choroid has many bright "tufts" (E). A single intravitreal injection of nanoceria (CeO_2) at P7 greatly inhibits the appearance of these "blebs" (C) and "tufts" (F). G and H show the quantitative analyses of IRN blebs and SRN tufts from this set of the experiment. Data were from nine animals, three at each of the three developmental ages (P14, P21and P28) with or without nanoceria. *p<0.05; **p<0.01. This figure (from Zhou *et al* 2011) was reproduced with the permission from PLoS One.

expression, prevent the development and maintenance of abnormal "leaky" intraretinal blood vessels (RNV) and inhibit choroidal neovascularizations (CNV) (Figures 1-3). They also decrease the levels of oxidative stress indicators (ROS, NADPH oxidase, nitrotyrosine, 8-hydrodeoxyguanosine) (Figure 4). These effects were still present 3 weeks after injection (161) without adverse toxicity or other side effects. Nanoceria also can down regulate VEGF expression levels and regress the existing vascular lesions in the Vldlr-/- mice when injected at P28 and analyzed at P35 (Figure 5) (161). These data demonstrated that nanoceria are effective in the treatment of pathologic neovascularizations in a mouse model of AMD and most likely will be as effective in humans with AMD, diabetic retinopathy (DR), retinopathy of prematurity (ROP) and other neovascular ocular diseases.

7. CONCLUSION AND PERSPECTIVES

AMD is a very complex and multifactor ocular disease. It has two clinical forms and has a wide variation in pathological development. In late stages of disease development, the dry form can change to the wet form and this phenomenon can also occur during treatment. Extensive efforts have been made to discover the etiology of AMD using molecular, cellular, biochemistry, physiology and animal models, yet the mechanisms underlying AMD are not fully known and will require further experimentation. We think, that because excessive production of ROS is a common node for degenerative diseases of the retina and because the rise in ROS occurs upstream of almost all other ocular pathology in AMD, it represents the "Achilles Heel" of AMD. Because nanoceria are catalytic antioxidants which are retained in the retina for months and act as a mixture of broad spectrum antioxidants to limit ROS and ROS-mediated damage, we think nanoceria can at least slow the progression of AMD and preserve vision in current patients with AMD. The combinatorial use of nanoceria with other therapeutic agents may lead to the most effective treatment
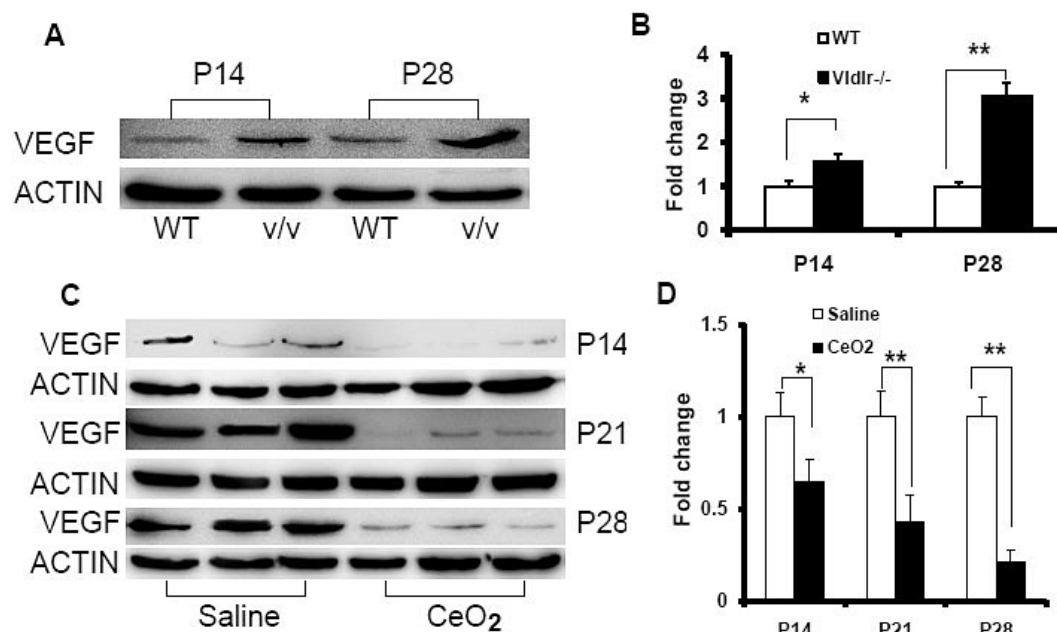
Figure 2. Nanoceria reduce the VEGF expression during the development of the Vldlr^{-/-} retina. Western blots show that VEGF protein levels are significantly higher in the Vldlr^{-/-} (v/v) retina than the wild type (WT) at P14 and P28 (A & B) and the increase is age-dependent. The developmental increases of VEGF levels are not altered by saline injection but nanoceria (CeO$_2$) injection at P7 significantly reduced the VEGF levels with maximum decreases of 5 fold (C & D). *p<0.05; **p<0.01. This figure (from Zhou *et al* 2011) was reproduced with the permission from PLoS One.
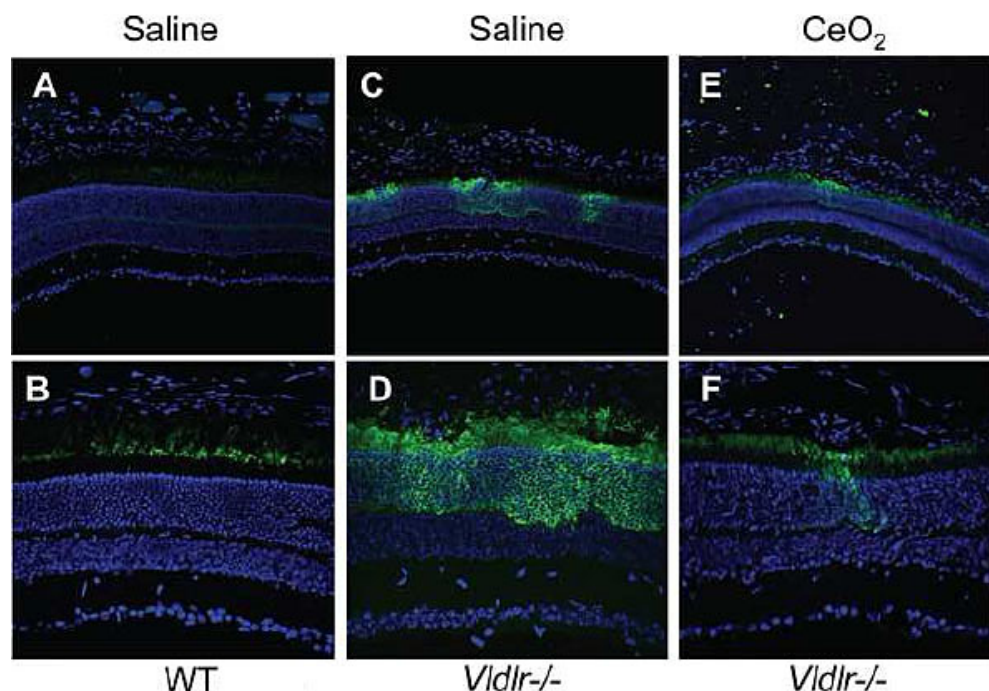
Figure 3. Nanoceria inhibit the ectopic expression of VEGF in the ONL of the Vldlr^{-/-} retina at P28. Photomicrographs of immunolocalization of VEGF in the Vldlr^{-/-} retina showed that WT retinas (A, B) had very low levels of VEGF in the ONL whereas discontinuous heavy staining of VEGF was located in the ONL of Vldlr^{-/-} (C, D). The labeling was greatly reduced in the nanoceria (CeO$_2$) injected (E, F) Vldlr^{-/-} mice. DAPI (blue) was used to visualize the nuclei. A, C, E, 20x; B, D, F, 40x. This figure (from Zhou *et al* 2011) was reproduced with the permission from PLoS One.
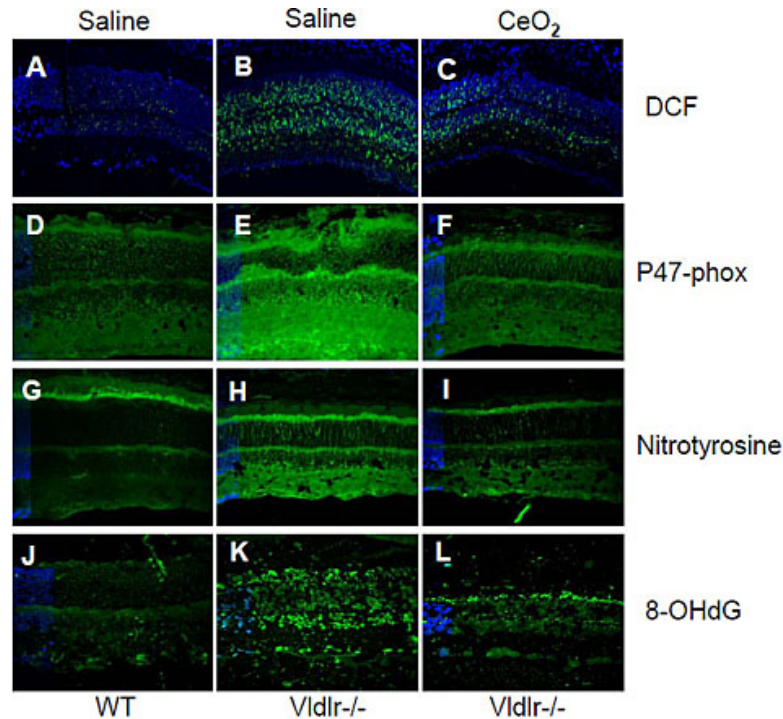
Figure 4. Nanoceria reduce oxidative stress in the Vldlr$^{-/-}$ retina. Eyes at P28 from wild type (left panel), Vldlr$^{-/-}$ injected with saline (middle panel) or nanoceria (CeO$_2$) (right panel) were sectioned and proceed for immunocytochemistry to visualize the distribution of oxidative stress markers for ROS (DCF), NADPH-oxidase (P47-phox), Nitrotyrosine and DNA damage (8-OHdG). Wild type shows either no labeling (A) or very low levels (D, G, J) of these markers, Vldlr$^{-/-}$ retina exhibits very high labeling (B, E, H, K) while nanoceria treatment greatly reduced the labeling of these markers (C, F, I, L). This figure (from Zhou *et al* 2011) was reproduced with the permission from PLoS One.
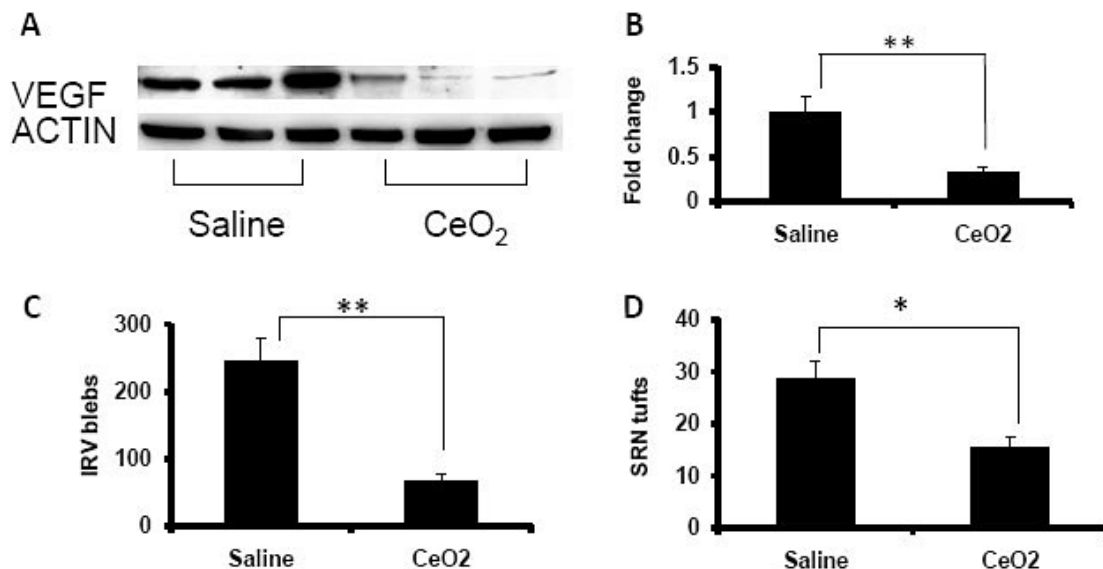
Figure 5. Retinal vascular lesions in the Vldlr$^{-/-}$ retinas require continual production of excess ROS. Vldlr$^{-/-}$ mice were injected at P28 with saline or nanoceria and killed one week later on P35. Analysis of VEGF levels by western blots (A) showed a four-fold reduction (B) within one week of nanoceria injection. The numbers of IRN blebs (C), and SRN tufts (D) were also dramatically reduced. *p<0.05; **p<0.01. This figure (from Zhou *et al* 2011) was reproduced with the permission from PLoS One.

for wet and dry AMD and other eye diseases which progress through the production of ROS.

9. REFERENCES

1. A. C. Bird: Age-related macular disease. *Br J Ophthalmol* 80, 2-3 (1996)

2. A. C. Bird: Therapeutic targets in age-related macular disease. *J Clin Invest* 120, 3033-41 (2010)

3. P. Elizabeth Rakoczy, M. J. Yu, S. Nusinowitz, B. Chang, J. R. Heckenlively: Mouse models of age-related macular degeneration. *Exp Eye Res* 82, 741-52 (2006)

4. A. O. Edwards, G. Malek: Molecular genetics of AMD and current animal models. *Angiogenesis* 10, 119-32 (2007)

5. G. Malek, L. V. Johnson, B. E. Mace, P. Saloupis, D. E. Schmechel, D. W. Rickman, C. A. Toth, P. M. Sullivan, C. Bowes Rickman: Apolipoprotein E allele-dependent pathogenesis: a model for age-related retinal degeneration. *Proc Natl Acad Sci* U S A 102, 11900-5 (2005)

6. H. R. Coleman, C. C. Chan, F. L. Ferris, 3rd, E. Y. Chew: Age-related macular degeneration. *Lancet* 372, 1835-45 (2008)

7. D. Zhu, X. Deng, J. Xu, D. R. Hinton: What determines the switch between atrophic and neovascular forms of age related macular degeneration? - the role of BMP4 induced senescence. *Aging* (Albany NY) 1, 740-5 (2009)

8. J. Xu, D. Zhu, S. He, C. Spee, S. J. Ryan, D. R. Hinton: Transcriptional regulation of bone morphogenetic protein 4 by tumor necrosis factor and its relationship with age-related macular degeneration. FASEB J 25, 2221-33 (2011)

9. X. Yuan, X. Gu, J. S. Crabb, X. Yue, K. Shadrach, J. G. Hollyfield, J. W. Crabb: Quantitative proteomics: comparison of the macular Bruch membrane/choroid complex from age-related macular degeneration and normal eyes. *Mol Cell Proteomics* 9, 1031-46 (2010)

10. R. Liutkeviciene, V. Lesauskaite, V. Asmoniene, D. Zaliuniene, V. Jasinskas: Factors determining age-related macular degeneration: a current view. *Medicina* (Kaunas) 46, 89-94 (2010)

11. B. S. Winkler, M. E. Boulton, J. D. Gottsch, P. Sternberg: Oxidative damage and age-related macular degeneration. *Mol Vis* 5, 32 (1999)

12. J. Liu, Y. Itagaki, S. Ben-Shabat, K. Nakanishi, J. R. Sparrow: The biosynthesis of A2E, a fluorophore of aging retina, involves the formation of the precursor, A2-PE, in the photoreceptor outer segment membrane. *J Biol Chem* 275, 29354-60 (2000)

13. D. H. Anderson, R. F. Mullins, G. S. Hageman, L. V. Johnson: A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol* 134, 411-31 (2002)

14. B. Zanke, S. Hawken, R. Carter, D. Chow: A genetic approach to stratification of risk for age-related macular degeneration. *Can J Ophthalmol* 45, 22-7 (2010)

15. S. Sivaprasad, N. V. Chong: The complement system and age-related macular degeneration. *Eye (Lond)* 20, 867-72 (2006)

16. G. S. Hageman, D. H. Anderson, L. V. Johnson, L. S. Hancox, A. J. Taiber, L. I. Hardisty, J. L. Hageman, H. A. Stockman, J. D. Borchardt, K. M. Gehrs, R. J. Smith, G. Silvestri, S. R. Russell, C. C. Klaver, I. Barbazetto, S. Chang, L. A. Yannuzzi, G. R. Barile, J. C. Merriam, R. T. Smith, A. K. Olsh, J. Bergeron, J. Zernant, J. E. Merriam, B. Gold, M. Dean, R. Allikmets: A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A* 102, 7227-32 (2005)

17. R. J. Klein, C. Zeiss, E. Y. Chew, J. Y. Tsai, R. S. Sackler, C. Haynes, A. K. Henning, J. P. SanGiovanni, S. M. Mane, S. T. Mayne, M. B. Bracken, F. L. Ferris, J. Ott, C. Barnstable, J. Hoh: Complement factor H polymorphism in age-related macular degeneration. *Science* 308, 385-9 (2005)

18. Z. Wu, T. W. Lauer, A. Sick, S. F. Hackett, P. A. Campochiaro: Oxidative stress modulates complement factor H expression in retinal pigmented epithelial cells by acetylation of FOXO3. *J Biol Chem* 282, 22414-25 (2007)

19. V. V. Lyzogubov, R. G. Tytarenko, P. Jha, J. Liu, N. S. Bora, P. S. Bora: Role of ocular complement factor H in a murine model of choroidal neovascularization. *Am J Pathol* 177, 1870-80 (2010)

20. J. Maller, S. George, S. Purcell, J. Fagerness, D. Altshuler, M. J. Daly, J. M. Seddon: Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. *Nat Genet* 38, 1055-9 (2006)

21. E. Gorgun, M. Guven, M. Unal, B. Batar, G. S. Guven, M. Yenerel, S. Tatlipinar, M. Seven, A. Yuksel: Polymorphisms of the DNA repair genes XPD and XRCC1

and the risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci* 51, 4732-7 (2010)

22. M. Kowalski, A. Bielecka-Kowalska, K. Oszajca, E. Makandjou-Ola, P. Jaworski, J. Bartkowiak, J. Szemraj: Manganese superoxide dismutase (MnSOD) gene (Ala-9Val, Ile58Thr) polymorphism in patients with age-related macular degeneration (AMD). *Med Sci Monit* 16, CR190-196 (2010)

23. F. Bonomini, F. Filippini, T. Hayek, M. Aviram, S. Keidar, L. F. Rodella, R. Coleman, R. Rezzani: Apolipoprotein E and its role in aging and survival. *Exp Gerontol* 45, 149-57 (2010)

24. S. Beatty, H. Koh, M. Phil, D. Henson, M. Boulton: The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 45, 115-34 (2000)

25. B. Halliwell: Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med* 91, 14S-22S (1991)

26. N. N. Osborne, T. A. Kamalden, A. S. Majid, S. del Olmo-Aguado, A. G. Manso, D. Ji: Light effects on mitochondrial photosensitizers in relation to retinal degeneration. *Neurochem Res* 35, 2027-34 (2010)

27. R. W. Young, D. Bok: Participation of the retinal pigment epithelium in the rod outer segment renewal process. *J Cell Biol* 42, 392-403 (1969)

28. B. Rohrer, Q. Long, B. Coughlin, B. Renner, Y. Huang, K. Kunchithapautham, V. P. Ferreira, M. K. Pangburn, G. S. Gilkeson, J. M. Thurman, S. Tomlinson, V. M. Holers: A targeted inhibitor of the complement alternative pathway reduces RPE injury and angiogenesis in models of age-related macular degeneration. *Adv Exp Med Biol* 703, 137-49 (2010)

29. Y. Totan, R. Yagci, Y. Bardak, H. Ozyurt, F. Kendir, G. Yilmaz, S. Sahin, U. Sahin Tig: Oxidative macromolecular damage in age-related macular degeneration. *Curr Eye Res* 34, 1089-93 (2009)

30. L. I. Lau, C. J. Liu, Y. H. Wei: Increase of 8-hydroxy-2'-deoxyguanosine in aqueous humor of patients with exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci* 51, 5486-90 (2010)

31. J. W. Crabb, M. Miyagi, X. Gu, K. Shadrach, K. A. West, H. Sakaguchi, M. Kamei, A. Hasan, L. Yan, M. E. Rayborn, R. G. Salomon, J. G. Hollyfield: Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A* 99, 14682-7 (2002)

32. A. Decanini, C. L. Nordgaard, X. Feng, D. A. Ferrington, T. W. Olsen: Changes in select redox proteins of the retinal pigment epithelium in age-related macular degeneration. *Am J Ophthalmol* 143, 607-15 (2007)

33. M. Suzuki, M. Kamei, H. Itabe, K. Yoneda, H. Bando, N. Kume, Y. Tano: Oxidized phospholipids in the macula increase with age and in eyes with age-related macular degeneration. *Mol Vis* 13, 772-8 (2007)

34. S. J. Fliesler, R. E. Anderson: Chemistry and metabolism of lipids in the vertebrate retina. *Prog Lipid Res* 22, 79-131 (1983)

35. R. E. Anderson, P. M. Lissandrello, M. B. Maude, M. T. Matthes: Lipids of bovine retinal pigment epithelium. Exp Eye Res 23, 149-57 (1976)

36. J. G. Hollyfield: Age-related macular degeneration: the molecular link between oxidative damage, tissue-specific inflammation and outer retinal disease: the Proctor lecture. *Invest Ophthalmol Vis Sci* 51, 1275-81 (2010)

37. J. G. Hollyfield, R. G. Salomon, J. W. Crabb: Proteomic approaches to understanding age-related macular degeneration. *Adv Exp Med Biol* 533, 83-9 (2003)

38. J. G. Hollyfield, V. L. Perez, R. G. Salomon: A hapten generated from an oxidation fragment of docosahexaenoic acid is sufficient to initiate age-related macular degeneration. *Mol Neurobiol* 41, 290-8 (2010)

39. J. R. Dias, E. B. Rodrigues, M. Maia, O. Magalhaes, Jr., F. M. Penha, M. E. Farah: Cytokines in neovascular age-related macular degeneration: fundamentals of targeted combination therapy. *Br J Ophthalmol* (2011)

40. C. N. Nagineni, V. K. Kommineni, A. William, B. Detrick, J. J. Hooks: Regulation of VEGF expression in human retinal cells by cytokines: implications for the role of inflammation in age-related macular degeneration. *J Cell Physiol* (2011)

41. R. A. Radu, J. Hu, Q. Yuan, D. L. Welch, J. Makshanoff, M. Lloyd, S. McMullen, G. H. Travis, D. Bok: Complement system dysregulation and inflammation in the retinal pigment epithelium of a mouse model for stargardt macular degeneration. *J Biol Chem* 286, 18593-601 (2011)

42. A. Salminen, A. Kauppinen, J. M. Hyttinen, E. Toropainen, K. Kaarniranta: Endoplasmic reticulum stress in age-related macular degeneration: trigger for neovascularization. *Mol Med* 16, 535-42 (2010)

43. M. Shimazawa, Y. Inokuchi, Y. Ito, H. Murata, M. Aihara, M. Miura, M. Araie, H. Hara: Involvement of ER stress in retinal cell death. *Mol Vis* 13, 578-87 (2007)

44. T. Sauer, M. Patel, C. C. Chan, J. Tuo: Unfolding the therapeutic potential of chemical chaperones for age-related macular degeneration. *Expert Rev Ophthalmol* 3, 29-42 (2008)

45. C. M. Ethen, C. Reilly, X. Feng, T. W. Olsen, D. A. Ferrington: The proteome of central and peripheral retina with progression of age-related macular degeneration. *Invest Ophthalmol Vis Sci* 47, 2280-90 (2006)

46. C. C. Chan, R. J. Ross, D. Shen, X. Ding, Z. Majumdar, C. M. Bojanowski, M. Zhou, N. Salem, Jr., R. Bonner, J. Tuo: Ccl2/Cx3cr1-deficient mice: an animal model for age-related macular degeneration. *Ophthalmic Res* 40, 124-8 (2008)

47. Y. Cho, X. Cao, D. Shen, J. Tuo, L. M. Parver, F. R. Rickles, C. C. Chan: Evidence for enhanced tissue factor expression in age-related macular degeneration. *Lab Invest* 91, 519-26 (2011)

48. A. A. Herzlich, J. Tuo, C. C. Chan: Peroxisome proliferator-activated receptor and age-related macular degeneration. *PPAR Res* 2008, 389507 (2008)

49. A. A. Herzlich, X. Ding, D. Shen, R. J. Ross, J. Tuo, C. C. Chan: Peroxisome Proliferator-Activated Receptor Expression in Murine Models and Humans with Age-related Macular Degeneration. *Open Biol J* 2, 141-148 (2009)

50. W. C. Wu, D. N. Hu, H. X. Gao, M. Chen, D. Wang, R. Rosen, S. A. McCormick: Subtoxic levels hydrogen peroxide-induced production of interleukin-6 by retinal pigment epithelial cells. *Mol Vis* 16, 1864-73 (2010)

51. V. Justilien, J. J. Pang, K. Renganathan, X. Zhan, J. W. Crabb, S. R. Kim, J. R. Sparrow, W. W. Hauswirth, A. S. Lewin: SOD2 knockdown mouse model of early AMD. *Invest Ophthalmol Vis Sci* 48, 4407-20 (2007)

52. N. B. Javitt, J. C. Javitt: The retinal oxysterol pathway: a unifying hypothesis for the cause of age-related macular degeneration. *Curr Opin Ophthalmol* 20, 151-7 (2009)

53. B. Dasari, J. R. Prasanthi, G. Marwarha, B. B. Singh, O. Ghribi: The oxysterol 27-hydroxycholesterol increases beta-amyloid and oxidative stress in retinal pigment epithelial cells. *BMC Ophthalmol* 10, 22 (2010)

54. K. S. Plafker, L. Nguyen, M. Barneche, S. Mirza, D. Crawford, S. M. Plafker: The ubiquitin-conjugating enzyme UbcM2 can regulate the stability and activity of the antioxidant transcription factor Nrf2. *J Biol Chem* 285, 23064-74 (2010)

55. S. M. Plafker: Oxidative stress and the ubiquitin proteolytic system in age-related macular degeneration. *Adv Exp Med Biol* 664, 447-56 (2010)

56. B. Medicherla, A. L. Goldberg: Heat shock and oxygen radicals stimulate ubiquitin-dependent degradation mainly of newly synthesized proteins. *J Cell Biol* 182, 663-73 (2008)

57. S. H. Byeon, S. C. Lee, S. H. Choi, H. K. Lee, J. H. Lee, Y. K. Chu, O. W. Kwon: Vascular endothelial growth factor as an autocrine survival factor for retinal pigment epithelial cells under oxidative stress via the VEGF-R2/PI3K/Akt. *Invest Ophthalmol Vis Sci* 51, 1190-7 (2010)

58. C. N. Roybal, L. Y. Marmorstein, D. L. Vander Jagt, S. F. Abcouwer: Aberrant accumulation of fibulin-3 in the endoplasmic reticulum leads to activation of the unfolded protein response and VEGF expression. *Invest Ophthalmol Vis Sci* 46, 3973-9 (2005)

59. J. C. Khan, D. A. Thurlby, H. Shahid, D. G. Clayton, J. R. Yates, M. Bradley, A. T. Moore, A. C. Bird: Smoking and age related macular degeneration: the number of pack years of cigarette smoking is a major determinant of risk for both geographic atrophy and choroidal neovascularisation. *Br J Ophthalmol* 90, 75-80 (2006)

60. M. Cano, R. Thimmalappula, M. Fujihara, N. Nagai, M. Sporn, A. L. Wang, A. H. Neufeld, S. Biswal, J. T. Handa: Cigarette smoking, oxidative stress, the anti-oxidant response through Nrf2 signaling, and Age-related Macular Degeneration. *Vision Res* 50, 652-64 (2010)

61. K. M. Bertram, C. J. Baglole, R. P. Phipps, R. T. Libby: Molecular regulation of cigarette smoke induced-oxidative stress in human retinal pigment epithelial cells: implications for age-related macular degeneration. *Am J Physiol Cell Physiol* 297, C1200-10 (2009)

62. M. Fujihara, N. Nagai, T. E. Sussan, S. Biswal, J. T. Handa: Chronic cigarette smoke causes oxidative damage and apoptosis to retinal pigmented epithelial cells in mice. *PLoS One* 3, e3119 (2008)

63. M. Pons, M. E. Marin-Castano: Cigarette Smoke-Related Hydroquinone Dysregulates MCP-1, VEGF and PEDF Expression in Retinal Pigment Epithelium in vitro and in vivo. *PLoS One* 6, e16722 (2011)

64. M. Pons, S. W. Cousins, K. G. Csaky, G. Striker, M. E. Marin-Castano: Cigarette smoke-related hydroquinone induces filamentous actin reorganization and heat shock protein 27 phosphorylation through p38 and extracellular signal-regulated kinase 1/2 in retinal pigment epithelium: implications for age-related macular degeneration. *Am J Pathol* 177, 1198-213 (2010)

65. J. Blasiak, A. Sklodowska, M. Ulinska, J. P. Szaflik: Iron and age-related macular degeneration. *Klin Oczna* 111, 174-7 (2009)

66. M. Hadziahmetovic, T. Dentchev, Y. Song, N. Haddad, X. He, P. Hahn, D. Pratico, R. Wen, Z. L. Harris, J. D. Lambris, J. Beard, J. L. Dunaief: Ceruloplasmin/hephaestin knockout mice model morphologic and molecular features of AMD. Invest Ophthalmol *Vis Sci* 49, 2728-36 (2008)

67. V. V. Lyzogubov, R. G. Tytarenko, J. Liu, N. S. Bora, P. S. Bora: Polyethylene Glycol (PEG)-induced Mouse Model of Choroidal Neovascularization. *J Biol Chem* 286, 16229-37 (2011)

68. H. L. Ramkumar, J. Zhang, C. C. Chan: Retinal ultrastructure of murine models of dry age-related macular

degeneration (AMD). *Prog Retin Eye Res* 29, 169-90 (2010)

69. L. L. Molday, A. R. Rabin, R. S. Molday: ABCR expression in foveal cone photoreceptors and its role in Stargardt macular dystrophy. *Nat Genet* 25, 257-8 (2000)

70. H. Sun, J. Nathans: Stargardt's ABCR is localized to the disc membrane of retinal rod outer segments. *Nat Genet* 17, 15-6 (1997)

71. J. Weng, N. L. Mata, S. M. Azarian, R. T. Tzekov, D. G. Birch, G. H. Travis: Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell* 98, 13-23 (1999)

72. N. L. Mata, J. Weng, G. H. Travis: Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. *Proc Natl Acad Sci U S A* 97, 7154-9 (2000)

73. H. Sun, R. S. Molday, J. Nathans: Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. *J Biol Chem* 274, 8269-81 (1999)

74. R. Allikmets, N. Singh, H. Sun, N. F. Shroyer, A. Hutchinson, A. Chidambaram, B. Gerrard, L. Baird, D. Stauffer, A. Peiffer, A. Rattner, P. Smallwood, Y. Li, K. L. Anderson, R. A. Lewis, J. Nathans, M. Leppert, M. Dean, J. R. Lupski: A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* 15, 236-46 (1997)

75. K. Jaakson, J. Zernant, M. Kulm, A. Hutchinson, N. Tonisson, D. Glavac, M. Ravnik-Glavac, M. Hawlina, M. R. Meltzer, R. C. Caruso, F. Testa, A. Maugeri, C. B. Hoyng, P. Gouras, F. Simonelli, R. A. Lewis, J. R. Lupski, F. P. Cremers, R. Allikmets: Genotyping microarray (gene chip) for the ABCR (ABCA4) gene. *Hum Mutat* 22, 395-403 (2003)

76. N. L. Mata, R. T. Tzekov, X. Liu, J. Weng, D. G. Birch, G. H. Travis: Delayed dark-adaptation and lipofuscin accumulation in abcr+/- mice: implications for involvement of ABCR in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 42, 1685-90 (2001)

77. L. Wu, T. Nagasaki, J. R. Sparrow: Photoreceptor cell degeneration in abcr (-/-) mice. *Adv Exp Med Biol* 664, 533-9 (2010)

78. M. P. Agbaga, R. S. Brush, M. N. Mandal, K. Henry, M. H. Elliott, R. E. Anderson: Role of Stargardt-3 macular dystrophy protein (ELOVL4) in the biosynthesis of very long chain fatty acids. *Proc Natl Acad Sci U S A* 105, 12843-8 (2008)

79. P. Tvrdik, R. Westerberg, S. Silve, A. Asadi, A. Jakobsson, B. Cannon, G. Loison, A. Jacobsson: Role of a new mammalian gene family in the biosynthesis of very long chain fatty acids and sphingolipids. *J Cell Biol* 149, 707-18 (2000)

80. M. P. Agbaga, M. N. Mandal, R. E. Anderson: Retinal very long-chain PUFAs: new insights from studies on ELOVL4 protein. *J Lipid Res* 51, 1624-42 (2010)

81. G. Karan, Z. Yang, K. Howes, Y. Zhao, Y. Chen, D. J. Cameron, Y. Lin, E. Pearson, K. Zhang: Loss of ER retention and sequestration of the wild-type ELOVL4 by Stargardt disease dominant negative mutants. *Mol Vis* 11, 657-64 (2005)

82. K. Zhang, M. Kniazeva, M. Han, W. Li, Z. Yu, Z. Yang, Y. Li, M. L. Metzker, R. Allikmets, D. J. Zack, L. E. Kakuk, P. S. Lagali, P. W. Wong, I. M. MacDonald, P. A. Sieving, D. J. Figueroa, C. P. Austin, R. J. Gould, R. Ayyagari, K. Petrukhin: A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy. *Nat Genet* 27, 89-93 (2001)

83. G. Karan, C. Lillo, Z. Yang, D. J. Cameron, K. G. Locke, Y. Zhao, S. Thirumalaichary, C. Li, D. G. Birch, H. R. Vollmer-Snarr, D. S. Williams, K. Zhang: Lipofuscin accumulation, abnormal electrophysiology, and photoreceptor degeneration in mutant ELOVL4 transgenic mice: a model for macular degeneration. *Proc Natl Acad Sci U S A* 102, 4164-9 (2005)

84. H. E. Grossniklaus, S. J. Kang, L. Berglin: Animal models of choroidal and retinal neovascularization. *Prog Retin Eye Res* 29, 500-19 (2010)

85. T. Tobe, S. Ortega, J. D. Luna, H. Ozaki, N. Okamoto, N. L. Derevjanik, S. A. Vinores, C. Basilico, P. A. Campochiaro: Targeted disruption of the FGF2 gene does not prevent choroidal neovascularization in a murine model. *Am J Pathol* 153, 1641-6 (1998)

86. J. R. Heckenlively, N. L. Hawes, M. Friedlander, S. Nusinowitz, R. Hurd, M. Davisson, B. Chang: Mouse model of subretinal neovascularization with choroidal anastomosis. *Retina* 23, 518-22 (2003)

87. W. Hu, A. Jiang, J. Liang, H. Meng, B. Chang, H. Gao, X. Qiao: Expression of VLDLR in the retina and evolution of subretinal neovascularization in the knockout mouse model's retinal angiomatous proliferation. *Invest Ophthalmol Vis Sci* 49, 407-15 (2008)

88. P. K. Frykman, M. S. Brown, T. Yamamoto, J. L. Goldstein, J. Herz: Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 92, 8453-7 (1995)

89. Y. Chen, Y. Hu, G. Moiseyev, K. K. Zhou, D. Chen, J. X. Ma: Photoreceptor degeneration and retinal inflammation induced by very low-density lipoprotein receptor deficiency. *Microvasc Res* 78, 119-27 (2009)

90. M. I. Dorrell, E. Aguilar, R. Jacobson, O. Yanes, R. Gariano, J. Heckenlively, E. Banin, G. A. Ramirez, M. Gasmi, A. Bird, G. Siuzdak, M. Friedlander: Antioxidant or neurotrophic factor treatment preserves function in a mouse model of neovascularization-associated oxidative stress. *J Clin Invest* 119, 611-623 (2009)

91. C. Li, Z. Huang, R. Kingsley, X. Zhou, F. Li, D. W. Parke, 2nd, W. Cao: Biochemical alterations in the retinas of very low-density lipoprotein receptor knockout mice: an animal model of retinal angiomatous proliferation. *Arch Ophthalmol* 125, 795-803 (2007)

92. Y. Chen, Y. Hu, K. Lu, J. G. Flannery, J. X. Ma: Very low density lipoprotein receptor, a negative regulator of the wnt signaling pathway and choroidal neovascularization. *J Biol Chem* 282, 34420-8 (2007)

93. A. Dong, B. Xie, J. Shen, T. Yoshida, K. Yokoi, S. F. Hackett, P. A. Campochiaro: Oxidative stress promotes ocular neovascularization. *J Cell Physiol* 219, 544-52 (2009)

94. Y. Imamura, S. Noda, K. Hashizume, K. Shinoda, M. Yamaguchi, S. Uchiyama, T. Shimizu, Y. Mizushima, T. Shirasawa, K. Tsubota: Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. Proc Natl Acad Sci U S A 103, 11282-7 (2006)

95. K. Hashizume, M. Hirasawa, Y. Imamura, S. Noda, T. Shimizu, K. Shinoda, T. Kurihara, K. Noda, Y. Ozawa, S. Ishida, Y. Miyake, T. Shirasawa, K. Tsubota: Retinal dysfunction and progressive retinal cell death in SOD1-deficient mice. Am J Pathol 172, 1325-31 (2008)

96. T. W. Mittag, A. U. Bayer, Vail Mm La: Light-induced retinal damage in mice carrying a mutated SOD I gene. Exp Eye Res 69, 677-83 (1999)

97. N. S. Bhise, R. B. Shmueli, J. C. Sunshine, S. Y. Tzeng, J. J. Green: Drug delivery strategies for therapeutic angiogenesis and antiangiogenesis. Expert Opin Drug Deliv 8, 485-504 (2011)

98. C. Campa, S. P. Harding: Anti-VEGF compounds in the treatment of neovascular age related macular degeneration. Curr Drug Targets 12, 173-81 (2011)

99. F. Enseleit, S. Michels, F. Ruschitzka: Anti-VEGF therapies and blood pressure: more than meets the eye. Curr Hypertens Rep 12, 33-8 (2010)

100. M. Friedlander: Combination angiostatic therapies: targeting multiple angiogenic pathways. Retina 29, S27-9 (2009)

101. U. Chakravarthy, J. Evans, P. J. Rosenfeld: Age related macular degeneration. BMJ 340, c981 (2010)

102. M. Caputo, H. Zirpoli, R. Di Benedetto, K. De Nadai, M. F. Tecce: Perspectives of choroidal neovascularization therapy. *Curr Drug Targets* 12, 234-42 (2011)

103. R. Hara, Y. Inomata, T. Kawaji, N. Sagara, M. Inatani, M. Fukushima, H. Tanihara: Suppression of choroidal neovascularization by N-acetyl-cysteine in mice. *Curr Eye Res* 35, 1012-20 (2010)

104. L. Zhao, B. V. Sweet: Lutein and zeaxanthin for macular degeneration. *Am J Health Syst Pharm* 65, 1232-8 (2008)

105. A. J. Chucair, N. P. Rotstein, J. P. Sangiovanni, A. During, E. Y. Chew, L. E. Politi: Lutein and zeaxanthin protect photoreceptors from apoptosis induced by oxidative stress: relation with docosahexaenoic acid. *Invest Ophthalmol Vis Sci* 48, 5168-77 (2007)

106. A. S. Prasad: Impact of the discovery of human zinc deficiency on health. *J Am Coll Nutr* 28, 257-65 (2009)

107. K. Kaarniranta, A. Salminen: NF-kappaB signaling as a putative target for omega-3 metabolites in the prevention of age-related macular degeneration (AMD). *Exp Gerontol* 44, 685-8 (2009)

108. J. Tuo, R. J. Ross, A. A. Herzlich, D. Shen, X. Ding, M. Zhou, S. L. Coon, N. Hussein, N. Salem, Jr., C. C. Chan: A high omega-3 fatty acid diet reduces retinal lesions in a murine model of macular degeneration. *Am J Pathol* 175, 799-807 (2009)

109. Z. Feng, Z. Liu, X. Li, H. Jia, L. Sun, C. Tian, L. Jia, J. Liu: alpha-Tocopherol is an effective Phase II enzyme inducer: protective effects on acrolein-induced oxidative stress and mitochondrial dysfunction in human retinal pigment epithelial cells. *J Nutr Biochem* 21, 1222-31 (2010)

110. R. Rastmanesh: Potential of melatonin to treat or prevent age-related macular degeneration through stimulation of telomerase activity. *Med Hypotheses* 76, 79-85 (2011)

111. R. A. Radu, N. L. Mata, S. Nusinowitz, X. Liu, P. A. Sieving, G. H. Travis: Treatment with isotretinoin inhibits lipofuscin accumulation in a mouse model of recessive Stargardt's macular degeneration. *Proc Natl Acad Sci U S A* 100, 4742-7 (2003)

112. R. A. Radu, Q. Yuan, J. Hu, J. H. Peng, M. Lloyd, S. Nusinowitz, D. Bok, G. H. Travis: Accelerated accumulation of lipofuscin pigments in the RPE of a mouse model for ABCA4-mediated retinal dystrophies following Vitamin A supplementation. *Invest Ophthalmol Vis Sci* 49, 3821-9 (2008)

113. A. Afzal, S. Caballero, S. S. Palii, S. Jurczyk, M. Pardue, D. Geroski, H. Edelhauser, G. Hochhaus, M. Kim, A. Franklin, G. Shapiro, M. B. Grant: Targeting retinal and choroid neovascularization using the small molecule

inhibitor carboxyamidotriazole. *Brain Res Bull* 81, 320-6 (2010)

114. O. Benny, K. Nakai, T. Yoshimura, L. Bazinet, J. D. Akula, S. Nakao, A. Hafezi-Moghadam, D. Panigrahy, P. Pakneshan, R. J. D'Amato: Broad spectrum antiangiogenic treatment for ocular neovascular diseases. *PLoS One* 5, e12515 (2010)

115. T. S. Griffith, T. Brunner, S. M. Fletcher, D. R. Green, T. A. Ferguson: Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 270, 1189-92 (1995)

116. J. Roychoudhury, J. M. Herndon, J. Yin, R. S. Apte, T. A. Ferguson: Targeting immune privilege to prevent pathogenic neovascularization. *Invest Ophthalmol Vis Sci* 51, 3560-6 (2010)

117. M. A. Economou, J. Wu, D. Vasilcanu, L. Rosengren, C. All-Ericsson, I. van der Ploeg, E. Menu, L. Girnita, M. Axelson, O. Larsson, S. Seregard, A. Kvanta: Inhibition of VEGF secretion and experimental choroidal neovascularization by picropodophyllin (PPP), an inhibitor of the insulin-like growth factor-1 receptor. *Invest Ophthalmol Vis Sci* 49, 2620-6 (2008)

118. Z. L. Chi, T. Yoshida, J. D. Lambris, T. Iwata: Suppression of drusen formation by compstatin, a peptide inhibitor of complement C3 activation, on cynomolgus monkey with early-onset macular degeneration. *Adv Exp Med Biol* 703, 127-35 (2010)

119. I. Ahmad, S. Balasubramanian, C. B. Del Debbio, S. Parameswaran, A. R. Katz, C. Toris, R. N. Fariss: Regulation of ocular angiogenesis by notch signaling: implications in neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci* 52, 2868-78 (2011)

120. M. Zheng, Z. Zhang, X. Zhao, Y. Ding, H. Han: The Notch signaling pathway in retinal dysplasia and retina vascular homeostasis. *J Genet Genomics* 37, 573-82 (2010)

121. T. Gridley: Notch signaling in the vasculature. *Curr Top Dev Biol* 92, 277-309 (2010)

122. A. K. Olsson, A. Dimberg, J. Kreuger, L. Claesson-Welsh: VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol* 7, 359-71 (2006)

123. H. Takahashi, H. Ishizaki, H. Tahara, Y. Tamaki, Y. Yanagi: Suppression of choroidal neovascularization by vaccination with epitope peptide derived from human VEGF receptor 2 in an animal model. Invest Ophthalmol Vis Sci 49, 2143-7 (2008)

124. H. Takahashi, R. Obata, Y. Tamaki: A novel vascular endothelial growth factor receptor 2 inhibitor, SU11248, suppresses choroidal neovascularization *in vivo*. *J Ocul Pharmacol Ther* 22, 213-8 (2006)

125. H. Takahashi, Y. Tamaki, N. Ishii, N. Oikawa, E. Mizuguchi, J. H. Francis, Y. Inoue, A. Iriyama, R. Obata, Y. Yanagi: Identification of a novel vascular endothelial growth factor receptor 2 inhibitor and its effect for choroidal neovascularization *in vivo*. *Curr Eye Res* 33, 1002-10 (2008)

126. L. Lu, B. C. Oveson, Y. J. Jo, T. W. Lauer, S. Usui, K. Komeima, B. Xie, P. A. Campochiaro: Increased expression of glutathione peroxidase 4 strongly protects retina from oxidative damage. *Antioxid Redox Signal* 11, 715-24 (2009)

127. S. M. Cashman, A. Desai, K. Ramo, R. Kumar-Singh: Expression of complement component 3 (c3) from an adenovirus leads to pathology in the murine retina. *Invest Ophthalmol Vis Sci* 52, 3436-45 (2011)

128. A. Davies, D. L. Simmons, G. Hale, R. A. Harrison, H. Tighe, P. J. Lachmann, H. Waldmann: CD59, an LY-6-like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. *J Exp Med* 170, 637-54 (1989)

129. N. S. Bora, P. Jha, V. V. Lyzogubov, S. Kaliappan, J. Liu, R. G. Tytarenko, D. A. Fraser, B. P. Morgan, P. S. Bora: Recombinant membrane-targeted form of CD59 inhibits the growth of choroidal neovascular complex in mice. *J Biol Chem* 285, 33826-33 (2010)

130. K. Ramo, S. M. Cashman, R. Kumar-Singh: Evaluation of adenovirus-delivered human CD59 as a potential therapy for AMD in a model of human membrane attack complex formation on murine RPE. *Invest Ophthalmol Vis Sci* 49, 4126-36 (2008)

131. A. Takeda, J. Z. Baffi, M. E. Kleinman, W. G. Cho, M. Nozaki, K. Yamada, H. Kaneko, R. J. Albuquerque, S. Dridi, K. Saito, B. J. Raisler, S. J. Budd, P. Geisen, A. Munitz, B. K. Ambati, M. G. Green, T. Ishibashi, J. D. Wright, A. A. Humbles, C. J. Gerard, Y. Ogura, Y. Pan, J. R. Smith, S. Grisanti, M. E. Hartnett, M. E. Rothenberg, J. Ambati: CCR3 is a target for age-related macular degeneration diagnosis and therapy. *Nature* 460, 225-30 (2009)

132. Y. Li, D. Huang, X. Xia, Z. Wang, L. Luo, R. Wen: CCR3 and Choroidal Neovascularization. *PLoS One* 6, e17106 (2011)

133. C. M. Lai, M. J. Estcourt, M. Wikstrom, R. P. Himbeck, N. L. Barnett, M. Brankov, L. B. Tee, S. A. Dunlop, M. A. Degli-Esposti, E. P. Rakoczy: rAAV.sFlt-1 gene therapy achieves lasting reversal of retinal neovascularization in the absence of a strong immune response to the viral vector. *Invest Ophthalmol Vis Sci* 50, 4279-87 (2009)

134. C. M. Lai, W. Y. Shen, M. Brankov, Y. K. Lai, N. L. Barnett, S. Y. Lee, I. Y. Yeo, R. Mathur, J. E. Ho, P. Pineda, A. Barathi, C. L. Ang, I. J. Constable, E. P. Rakoczy: Long-term evaluation of AAV-mediated sFlt-1 gene therapy for ocular neovascularization in mice and monkeys. *Mol Ther* 12, 659-68 (2005)

135. J. Tuo, J. J. Pang, X. Cao, D. Shen, J. Zhang, A. Scaria, S. C. Wadsworth, P. Pechan, S. L. Boye, W. W. Hauswirth, C. C. Chan: AAV5-mediated sFLT01 gene therapy arrests retinal lesions in Ccl2(-/-)/Cx3cr1(-/-) mice. *Neurobiol Aging* (2011)

136. Y. Huang, V. Enzmann, S. T. Ildstad: Stem cell-based therapeutic applications in retinal degenerative diseases. *Stem Cell Rev* 7, 434-45 (2011)

137. T. J. Rowland, D. E. Buchholz, D. O. Clegg: Pluripotent human stem cells for the treatment of retinal disease. *J Cell Physiol* (2011)

138. R. E. MacLaren, R. A. Pearson, A. MacNeil, R. H. Douglas, T. E. Salt, M. Akimoto, A. Swaroop, J. C. Sowden, R. R. Ali: Retinal repair by transplantation of photoreceptor precursors. Nature 444, 203-7 (2006)

139. V. Marchetti, T. U. Krohne, D. F. Friedlander, M. Friedlander: Stemming vision loss with stem cells. J Clin Invest 120, 3012-21 (2010)

140. G. A. Silva, N. M. Silva, T. M. Fortunato: Stem cell and tissue engineering therapies for ocular regeneration. Curr Stem Cell Res Ther 6, 255-72 (2011)

141. D. Zhu, X. Deng, C. Spee, S. Sonoda, C. L. Hsieh, E. Barron, M. Pera, D. R. Hinton: Polarized Secretion of PEDF from Human Embryonic Stem Cell-Derived RPE Promotes Retinal Progenitor Cell Survival. Invest Ophthalmol Vis Sci 52, 1573-85 (2011)

142. A. Otani, K. Kinder, K. Ewalt, F. J. Otero, P. Schimmel, M. Friedlander: Bone marrow-derived stem cells target retinal astrocytes and can promote or inhibit retinal angiogenesis. Nat Med 8, 1004-10 (2002)

143. J. Lecomte, K. Louis, B. Detry, S. Blacher, V. Lambert, S. Bekaert, C. Munaut, J. Paupert, P. Blaise, J. M. Foidart, J. M. Rakic, S. M. Krane, A. Noel: Bone marrow-derived mesenchymal cells and MMP13 contribute to experimental choroidal neovascularization. Cell Mol Life Sci 68, 677-86 (2011)

144. H. Y. Hou, H. L. Liang, Y. S. Wang, Z. X. Zhang, B. R. Wang, Y. Y. Shi, X. Dong, Y. Cai: A therapeutic strategy for choroidal neovascularization based on recruitment of mesenchymal stem cells to the sites of lesions. Mol Ther 18, 1837-45 (2010)

145. M. Tomita, H. Yamada, Y. Adachi, Y. Cui, E. Yamada, A. Higuchi, K. Minamino, Y. Suzuki, M. Matsumura, S. Ikehara: Choroidal neovascularization is provided by bone marrow cells. Stem Cells 22, 21-6 (2004)

146. K. M. Farjo, J. X. Ma: The potential of nanomedicine therapies to treat neovascular disease in the retina. *J Angiogenes Res* 2, 21 (2010)

147. X. Cai, S. Conley, M. Naash: Nanoparticle applications in ocular gene therapy. *Vision Res* 48, 319-24 (2008)

148. R. Ellis-Behnke, J. B. Jonas: Redefining tissue engineering for nanomedicine in ophthalmology. *Acta Ophthalmol*. 89 e108-14 (2011)

149. S. Tsunekawa, R. Sahara, Y. Kawazoe, K. Ishikawa: Lattice relaxation of manosize CeO2-X nanocrystalline particles. *Applied Surface Science* 152, 53-56 (1999)

150. A. S. Karakoti, S. Singh, A. Kumar, M. Malinska, S. V. Kuchibhatla, K. Wozniak, W. T. Self, S. Seal: PEGylated nanoceria as radical scavenger with tunable redox chemistry. *J Am Chem Soc* 131, 14144-5 (2009)

151. J. Chen, S. Patil, S. Seal, J. F. McGinnis: Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. *Nat Nanotechnol* 1, 142-50 (2006)

152. A. Vincent, T. M. Inerbaev, S. Babu, A. S. Karakoti, W. T. Self, A. E. Masunov, S. Seal: Tuning hydrated nanoceria surfaces: experimental/theoretical investigations of ion exchange and implications in organic and inorganic interactions. *Langmuir* 26, 7188-98 (2010)

153. J. M. Perez, A. Asati, S. Nath, C. Kaittanis: Synthesis of biocompatible dextran-coated nanoceria with pH-dependent antioxidant properties. Small 4, 552-6 (2008)

154. E. G. Heckert, A. S. Karakoti, S. Seal, W. T. Self: The role of cerium redox state in the SOD mimetic activity of nanoceria. Biomaterials 29, 2705-9 (2008)

155. J. Chen, S. Patil, S. Seal, J. F. McGinnis: Nanoceria particles prevent ROI-induced blindness. Adv Exp Med Biol 613, 53-9 (2008)

156. S. M. Hirst, A. S. Karakoti, R. D. Tyler, N. Sriranganathan, S. Seal, C. M. Reilly: Anti-inflammatory properties of cerium oxide nanoparticles. Small 5, 2848-56 (2009)

157. N. Link, T. J. Brunner, I. A. Dreesen, W. J. Stark, M. Fussenegger: Inorganic nanoparticles for transfection of mammalian cells and removal of viruses from aqueous solutions. Biotechnol Bioeng 98, 1083-93 (2007)

158. B. K. Pierscionek, Y. Li, A. A. Yasseen, L. M. Colhoun, R. A. Schachar, W. Chen: Nanoceria have no genotoxic effect on human lens epithelial cells. Nanotechnology 21, 035102 (2010)

159. L. L. Wong, Q. N. Pye, S. M. Hirst, X. Cai, C. M. Reilly, S. Seal, J. F. McGinnis: Pharmacokinetics and

effects of nanoceria in normal and P23H degenerative rat retinas. ARVO abstract #3416 (2011)

160. L. Kong, X. Cai, X. Zhou, L. L. Wong, A. S. Karakoti, S. Seal, J. F. McGinnis: Nanoceria extend photoreceptor cell lifespan in tubby mice by modulation of apoptosis/survival signaling pathways. Neurobiol Dis 42, 514-23 (2011)

161. X. Zhou, L. L. Wong, A. S. Karakoti, S. Seal, J. F. McGinnis: Nanoceria inhibit the development and promote the regression of pathologic retinal neovascularization in the vldlr knockout mouse. *PLoS One* 6, e16733 (2011)

Abbreviations: AMD: age-related macular degeneration, BM: Bruch's membrane, RPE: retinal pigment epithelium, CNV: choroidal neovascularization, BMP4: bone morphogenetic protein-4, TNF: tumor necrosis factor, SNP: single nucleotide polymorphisms, C3: complement component 3, MAC: membrane attack complex, CHF: complement factor H, MIRL: membrane inhibitor of reactive lysis, FOXO3: forkhead box, type O, member 3, XPD: xeroderma pigmentosum complementation group D, MnSOD: manganese superoxide dismutase, ApoE: apolipoprotein E, HF-C: high fat cholesterol-rich, TR: targeted replacement, RNV: retinal neovescularization, Abeta: beta-amylopid, ROS: Reactive oxygen species, H_2O_2: hydrogen peroxide, OS: outer segment, PUFAs: polyunsaturated fatty acids, MDA: malondialdehyde, PC: protein carbonyl, 8-OHdG: 8-hydroxy-29-deoxygianosine, TOS: total oxidation status, TAC: total antioxidant capacity, DHA: docosahexaenoic acid, CEP: carboxyethylpyrrole, IL-1: interleukin-1, IFN: interferon, PDGF: platelet-derived growth factor, FGF: fibroblast growth factor, A2E: N-retinylidene-N-retinylethanomalmine, VEGF: endothelial growth factor, ER: endoplasmic reticulum, IRE1: inositol-requiring protein-1, PERK: protein kinase RNA-like ER kinase, ATF6: activating transcription factor-6, UPR: unfolded protein response, CHOP: CCAAT/enhancer-binding protein homologous protein, HSP60: heat shot protein 60, HOP: Hsp70/Hsp90 organizing protein, Ccr2$^{-/-}$/Cx3cr1$^{-/-}$: chemokine CCL receptor (CCR2) and chemokine C-X3-C receptor 1 (CX3CR1) double knockout, TF: tissue factor, LPS: lipopolysaccharide, PPARs: peroxisome proliferator-activated receptors, MMPs: matrix metalloproteases, HO-1: heme-oxygenase-1, IL-6: interleukin-6, SOD2: super oxide dismutase 2, 27-HOC: 27-hydroxycholesterol, NF-kB: nucleus factor-kappaB, UPS: ubiquitin proteolytic system, Nrf2: nuclear factor erythroid-derived 2, like 2, HQ: hydroquinone, 4-HNE: 4-hydroxy-2-nonenal, GSH: glutathione, MCP: monocyte chemoattractant protein, PEDF: pigment epithelium-derived factor, Hsp27: heat shock protein 27, ERK: extracellular signal-regulated kinase, Cp: ceruolasmin, Heph: hephaestin, PEG-8: polyethylene glycol-8, TGF: transforming growth factor, bFGF: basic fibroblast growth factor, ABCA4: ATP-binding cassette transporter sub-family A member 4, Abcr$^{-/-}$: Abcr knockout mouse, N-ret-PE: N-retinylidene phosphatidylethanolamine, CRPs: complement-regulatory proteins , ELO: elongation of long chain fatty acid, VLC-FA : very long chain saturated fatty acids, VLC-PUFA: very long chain polyunsaturated fatty acids, Vldlr: very low density lipoprotein receptor, RAP: retinal angiomatous proliferation, P: postnatal day, GFAP: glial fibrillary acidic protein, LRP5/6: low-density lipoprotein receptor-related protein 5 or 6, wnt: wg/int1, wingless/integration 1, Sod1: superoxide dismutase 1, ONL: outer nuclear layer, INL: inner nuclear layer, WT: wild type, FDA: US Food and Drug Administration, CXL: chemokine (C-X-C motif) ligand, VEGFR: VEGF receptor, LUT: lutein, ZEA: zeaxanthin, BC: beta-carotene, Omega-3 LCPUFA: long-chain omega-3 polyunsaturated (n-3) fatty acids, CAI: carboxyamidotriazole, bHPCD-CAI: beta-hydroxypropyl cyclodextrin, IGF-1R: Insulin-like growth factor-1 receptor, PPP: cyclolignan picropodophyllin, Gpx 4: glutathione peroxidase 4, CAP: complement alternative pathway, CR2: complement receptor 2, hCD59: human CD59, Ad: adenovirus, CCR3: eosinophil/mast cell chemokine receptor, AAV-sFlt: AAV-mediated soluble form of VEGF receptor, GFP: green florescent protein, rds: retinal degeneration slow, rd: retinal degeneration, rho-/-: rhodopsin knockout, hES-RPE: Human embryonic stem cell-derived RPE, RPCs: retinal progenitor cells, EPCs: endothelial progenitor cells, MMP: matrix metalloproteinase, MSCs: mesenchymal stem cells, EGFP: enhanced green fluorescent protein, Nanoceria: cerium oxide nanoparticles, ICP-MS: inductively coupled plasma-mass spectrometry, DR: diabetic retinopathy, ROP: retinopathy of prematurity

Send correspondence to: James F. McGinnis, Department of Ophthalmology, Dean McGee Eye Institute, Department of Cell Biology and Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, Tel: 405-271-3695, Fax, 405-271-3721, E-mail: james-mcginnis@ouhsc.edu