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Models of retinal diseases and their applicability in drug discovery

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Abstract

Introduction: The impact of vision debilitating diseases is a global public health concern, which will continue until effective preventative and management protocols are developed. Two retinal diseases responsible for the majority of vision loss in the working age adults and elderly populations are diabetic retinopathy (DR) and age-related macular degeneration (AMD), respectively. Model systems, which recapitulate aspects of human pathology, are valid experimental modalities that have contributed to the identification of signaling pathways involved in disease development and consequently potential therapies.

Areas covered: The pathology of DR and AMD, which serve as the basis for designing appropriate models of disease, is discussed. The authors also review *in vitro* and *in vivo* models of DR and AMD and evaluate the utility of these models in exploratory and pre-clinical studies.

Expert opinion: The complex nature of non-Mendelian diseases such as DR and AMD has made identification of effective therapeutic treatments challenging. However, the authors believe that while *in vivo* models are often criticized for not being a ‘perfect’ recapitulation of disease, they have been valuable experimentally when used with consideration of the strengths and limitations of the experimental model selected and have a place in the drug discovery process.

Keywords

age-related macular degeneration; animal models; cell culture models; diabetic retinopathy

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1. Introduction

The eye is an intricate and extraordinary organ able to translate visual light into biological signals, which are then interpreted by the brain [1]. Its sophisticated design and function during development, aging, and disease, can be influenced by multiple factors including genetics, environment, and lifestyle, all of which may play an important role in contributing to its complexity at each stage. This is clearly exemplified by retinal diseases, which have the undesirable effect of compromising vision, to varying degrees, including total blindness. Two retinal diseases responsible for a large fraction of new vision loss in working age adults (20–74 years of age) and elderly individuals (>60 years) are diabetic retinopathy (DR) and age-related macular degeneration (AMD), respectively [2,3]. Currently, it is estimated that world-wide there are approximately 126.6 million people affected by DR and an additional 187 million living with AMD [4,5]. Though treatment options are available for DR and AMD patients, they are limited, and efficacy varies significantly amongst patients. This highlights the need for identification of not only new therapeutic targets, but also animal models that present with pathology paralleling that seen in human patients.

Models of retinal diseases have been not only instrumental to identification of potential new signaling pathways involved in initiation and progression of disease, but also serve as platforms to test new therapies. Both *in vitro* and *in vivo* models have been successfully used as necessary pre-clinical modalities to test new therapies and have served as the launching point, providing compelling evidence, for initiation of clinical trials [6,7]. In spite of this, frequently, animal models of complex diseases are rejected, as they are perceived as not fully recapitulating the human condition. Arguably, creating the ‘perfect’ model of a complex disease, though laudable, may be improbable, given patients with diseases such as DR or AMD do not express all the risk factors identified to date, nor do they exhibit all the clinical phenotypic features of the disease. Herein we will review recent advances in the development of *in vitro* models that may be used for high through put screening of drugs as well as *in vivo* models that develop key phenotypic characteristics of DR and AMD.

2. Diabetic retinopathy (DR)

Currently 300 million individuals have diabetes worldwide and the prevalence is rising rapidly. Over one-third of diabetic individuals will develop DR and approximately 10% of these individuals will suffer from vision threatening disease. DR progresses in many individuals despite preventable measures such as good blood sugar, blood pressure and serum lipid control. DR pathogenesis is multifactorial including leukocyte involvement, basement membrane thickening and pericyte and endothelial loss. Early on there is vasodegeneration of capillaries that leads to retinal ischemia and non-perfusion. In addition to vascular damage the neuronal cells of the retina become compromised even before there is clear evidence of vascular compromise. While the histological and fundusoscopic features are well-characterized in humans and animals models of the disease, the mechanisms involved remain incompletely understood [8].

3. *In vitro* tools for modeling DR

Clinical manifestations of DR are microvascular, therefore historically research studies have focused on endothelial and pericyte cell culture models. As it became clear that neuroglia, as well as retinal pigment epithelial cells (RPE) contribute to the development of the disease, DR models of Müller cells, microglia, ganglion cells and RPE cells, as well as co-culture systems were developed and are now commonly used. In addition, cell culture models to determine the role of bone marrow-derived cells in retinal vascular damage and repair are used in DR research (Table 1). Below, each of these models will be discussed.

3.1 Retinal Endothelial Cells (REC)

REC isolated from bovine (BREC) [9–26] and human donor eyes (HREC) [21,27–50] are used in a significant number of studies, followed by porcine [23,51,52], rhesus monkey [53,54], feline [55], rat [56] and mouse REC [57,58]. REC cultures from bovine and human eyes are obtained by retinal digestion and filter separation of the microvessels followed by homogenization, digestion and further purification by gradient centrifugation, antibody-based purification [45], or selective culture conditions optimized for each species [34,59,60]. Porcine [23,51,52], rhesus monkey [53,54], feline [55] and rat [61] REC are isolated and cultured similar to bovine and human, however mouse cultures are notoriously difficult potentially due to different mouse osmolarity and sparse amount of starting material. The only mouse endothelial cell culture reported to date is from platelet endothelial cell adhesion molecule-1 (PECAM-1) antibody based purification of retinas isolated from immortomice [58].

To study the effect of diabetes on REC several experimental paradigms are used. Most of them involve treatment of normal control REC with diabetogenic media. The composition of diabetogenic media varies depending on the study and could include high glucose [27,46,62–66], saturated and omega 6 polyunsaturated fatty acids [67–71], ceramide, cholesterol [68,72], cytokines [42,50,68,72,73] and vascular endothelial growth factor (VEGF) [9,74] treatments. Exposure of endothelial cells to hypoxic conditions [75], or treatment with CoCl₂ to induce pseudohypoxia [76] are also used. Recently the phenomenon of metabolic memory, or legacy effect was discovered and it was demonstrated that HREC isolated from diabetic patients or animal models maintain the diabetic phenotype for several passages [34,36,77–80]. HREC isolated from control and diabetic donors have also become a valuable model to examine the effects of diabetes as a whole on REC damage and repair [34,36].

Using these experimental paradigms several endpoints for the effects of diabetes and diabetogenic media on REC are tested.

Permeability—Retinal microvascular endothelial cells are part of the blood-retinal barrier and breakdown of this barrier with an increase in retinal vascular permeability are amongst the earliest diabetes-induced changes in DR. It is thus important to have reliable cell culture models to understand the effects of the diabetic metabolic abnormalities on endothelial cell barrier function. The most well characterized model for these studies are BREC [9,74]. BREC form uniform monolayers connected by tight junctions with electrical resistance

above 200Ω/cm². They also respond to VEGF and cytokines with increased permeability [9,74,81]. This model has been used to demonstrate the role of pro-inflammatory and pro-angiogenic factors in diabetes-induced tight junction abnormalities. Additionally, though HREC monolayers are leaky as they do not form a tight barrier, they can be used to determine the molecular mechanisms of the effects of diabetes on tight junction proteins [35,82,83].

Pro-inflammatory changes—Inflammation and increase in inflammatory cytokines are well accepted to play an important role in the pathogenesis of DR. Treatment of BREC and HREC with cytokines known to be increased in the diabetic retina, IL-1β and TNFα, followed by NFκB pathway activation leads to an increase in adhesion molecules ICAM-1 (intercellular) and VCAM-1 (vascular), and leukocyte adhesion [27,33,36,43,72,73,84–89]. Similar effects have also been shown after the exposure to pro-inflammatory omega 6 fatty acids [30]. These effects may be blocked by decreasing the levels of cholesterol or ceramide in the endothelial cell membrane [30,36,42,72,90]. There is a lot of controversy in the literature on the role of high glucose in controlling REC inflammatory state. Several studies have demonstrated that high glucose alone is a very weak stimulus for pure endothelial cells and that the high glucose response of endothelial cells is driven by other cell types normally present at lower levels in endothelial cell cultures, such as microglia and Müller cells [27,30]. However, most of the studies show a slight upregulation of pro-inflammatory, pro-apoptotic factors, and mitochondrial damage after prolonged exposure to high glucose [12,14,27,32,35,47,65,91–96].

Apoptosis—Endothelial cell apoptosis leads to formation of acellular capillaries, the hallmark of DR, in the retina. The role of high glucose, cytokines, fatty acids, cholesterol, sphingolipids, oxidative stress and mitochondrial damage is widely studied using BREC and HREC cultures. Endothelial cells are shown to have both intrinsic and extrinsic apoptotic pathways with activation of cytochrome C release from mitochondria and Caspase 9, Caspase 8 and FLIP pathways leading to classical Caspase 3/7 effector caspases activation and execution of apoptosis [13,20,28,92,97–101].

Tube formation—Tube formation assay is used to access both normal migratory, proliferative and repair capacity of REC, as well as REC response to neovascular stimuli. HREC isolated from diabetic donor tissue, as well as HREC and BREC treated with diabetogenic media have been shown to have defective tube formation that could be improved by inhibition of inflammatory or apoptotic factors, or introduction of reparative progenitor cells as will be discussed later [36].

3.2 Pericytes

Pericyte dropout is one of the earliest features of DR and the role of pericytes in support of retinal microvascular endothelial cell barrier through both mechanical connection as well as secretion of paracrine factors is well accepted. Pericytes isolated from human, bovine and murine tissue are widely used in DR pathway and drug discovery research. Pericytes isolated from control and diabetic tissue, as well as treated with high glucose, oxidized glycated low density lipoproteins (LDL), and cytokines are used to study the role of autophagy and ER

stress in pericyte apoptosis, and paracrine factors secreted by pericytes, including ATP release and activation of P2X7R [102–108]. In addition, the role of chemomechanical contraction of pericytes in endothelial cell function can be assessed using pericytes isolated from control and diabetic donor tissue, as well as pericytes treated with high glucose, lipids and cytokines [109].

3.3 Müller cells

Müller cells are the primary glia cells in the retina that span the whole thickness of the retina and, like pericytes, have direct contact with endothelial cells, as well as neuroretina. Loss of Müller cells protective paracrine factors and increase of pro-inflammatory cytokine production by Müller cells in diabetes is studied using several cell culture models, including Müller cells isolated from human donor tissue, as well as human and rat Müller cell lines. Using these models, the role of hyperglycemia, oxidized LDL, and K channels in cytokine and VEGF production has been evaluated [110–122].

3.4 Microglia

Microglial activation occurs early in DR and is proposed to contribute to the pro-inflammatory status and pathogenesis of DR [36,123,124]. There are very few studies using microglia cell culture models of DR due to several factors. First of all, the markers that are used to identify and isolate microglia (Iba-1, CD11b, CD68) are shared between microglia and other monocytes making it challenging to separate pure populations of resident microglia from extravasated immune cells. Moreover, newborn murine retinas are required for viable cultures of microglia making it very difficult to obtain enough starting retinal material for experimental treatments [125].

3.5 RPE cells

The retinal pigment epithelial layer is known for its role in diabetic macular edema, as loss of pigment epithelial-derived factor (PEDF), a trophic factor produced by RPE cells, has been shown to contribute to pericyte loss and endothelial damage in DR. Thus RPE cell cultures are important tools in DR research. It is advantageous that human, bovine, porcine and murine RPE cells are relatively easy to isolate and grow in culture. Additionally, although not without important limitations, the spontaneously immortalized RPE cell line, ARPE-19 [126], has been used extensively in RPE cell research. This RPE cell model has been reported to have low barrier function due to rudimentary tight junctions and variable expression of claudins [127]. However, it maintains a number of characteristics of human RPE cells, not all, is easy to grow in culture, and provides a useful and convenient model for DR and macula edema research.

Multiple studies have examined the effect of metabolic factors, including high glucose and lipids on glucose transport and glucose metabolism in RPE cells [128–135]. RPE cells in culture have been shown to have high levels of aldose reductase [136]. High glucose conditions lead to an increase in aldose reductase activity in RPE cells [136], and inhibition of aldose reductase prevented oxidative stress, cytokine production and apoptosis in high glucose treated RPE cells [136]. The utility of RPE cells has further been demonstrated in

studies of PEDF, which have confirmed the role of diabetogenic conditions in inhibition of the production of this trophic factor using RPE cell culture models [128,137,138].

3.6 Ganglion cells

Ganglion cell death is a well accepted part of DR pathogenesis and production of VEGF by ganglion cells in response to succinate has been shown to contribute to inner blood retina barrier (BRB) breakdown in DR. Early papers used retinal ganglion cell line 5 (RGC5) to study the effects of hyperglycemia on ganglion cells, however the origin of RGC5 cells was later questioned and studies on this cell line were not verified [139]. Ganglion cells isolated from embryonic rat retinas by panning protocol are viable and can be obtained at high purity [140]. These cells have been used to determine the role of TNF α [141], PEGF [142], metabolic activity [143], and sigma receptor 1 [144] as a model of diabetes-induced damage.

3.7 Photoreceptor cells

Recent studies have revealed the contribution of photoreceptor damage to DR pathogenesis. Although there are several models for “shaving” or isolating photoreceptors, they are notoriously difficult, and the cells do not grow in culture. Most of the cell culture studies rely on a mouse SV-40 T antigen transformed photoreceptor cell line 661W [145]. The effect of hypoglycemia on photoreceptor cells has been tested using this model [146].

3.8 Co-culture studies

As the retina is a complex organ, most of the effects involve multiple cell types. To capture this complexity several co-culture models have been applied. These include media transfer models where pericytes, Müller or RPE cells are cultured in high glucose or lipid mediators and the media with the factors produced by these cells is used to treat endothelial cells. Co-culture of pericytes and endothelial cells has also been used to test the role of cell-cell interactions in fluctuating high/low glucose-induced breakdown on permeability barrier [147] and to test chemomechanical contraction of pericytes in endothelial cell proliferation [109,148].

In addition to retinal cells, bone marrow-derived circulating angiogenic cells (CACs) are known to both incorporate into the vessels and produce paracrine factors that aid in endothelial repair. Co-culture models of HREC isolated from control and diabetic patients combined with CD34⁺ CACs isolated from control and diabetic donors may be used to develop the strategies for improved cell-based therapy for DR [36].

4. *In vivo* models of DR

There are many animal models of diabetes. While it is well known that rodent models develop only the early stages of DR, they still remain the most popular models. This is largely because of their cost and well-described histological and functional characteristics. In addition to the economic considerations, rodent models have the benefit of their short generation time. In this section, selected and highly popular models will be briefly described with the specific intent of describing the histological and functional features of the model that can be reliably used to validate a pharmacological intervention, taking into account the

models advantages/disadvantages. From the perspective of pre-clinical efficacy, a model must have key DR endpoints and that the “endpoints” have a reproducible time line. Specific models include type 1 diabetes (T1D) and type 2 diabetes (T2D) models and models that have “mixed” features such as the type-2 diabetes in rats that is induced by a high fat diet and streptozotocin (STZ).

Regarding the endpoints, perhaps the “classic” gold standard of DR is enumeration of acellular capillaries. This histological endpoint is representative of vasodegeneration and considers both the loss of pericytes and endothelial cells. While the pathology implicit in this histological endpoint is the culmination of many deleterious events including inflammatory cell activation, enhanced cytokine expression, oxidative injury and many more. Acellular capillaries are the “hallmark” feature of DR. Prior to popular use of acellular capillaries, the enumeration of pericyte loss (pericyte ghosts) was also considered an early and reproducible event in DR. Similarly, vascular permeability is a critical and assessable endpoint for DR [149]. A model that recapitulates many of the histological features of DR is the model of ischemia/reperfusion [150]. This model has acellular capillaries and increases in permeability (Figure 1). Although ischemia/reperfusion is different from diabetes-induced pathogenesis as it does not involve hyperglycemic environment, the endpoints of this easy economical model provide a fast way to evaluate retinal vascular changes and to test interventions.

Diabetes models are generated typically through drugs, diet, or chemical damage. Genetic models are produced by selective breeding and gene editing. Mice have been the emphasis of most genetic studies, with the discovery of inherited obesity or hyperglycemia resulting in a diabetic phenotype [151–153]. However, other species have advances over rodent DR models. As put forth by Engerman, dog models appear to be most similar to human DR [154]. Pigs are preferred for the similarity of their eye structure to humans and Zebra fish for their short life span and large breeding sizes [155]. Unfortunately, nonhuman primates have proven relatively resistant to induced DR [156]. Although no single animal model represents the complete range of vascular and neural complications of human DR from both early and late stages, the murine models described below and summarized in Table 2 have been instrumental in establishing mechanisms responsible for DR and have provided reproducible pre-clinical data.

4.1 Drug-induced models of DR

Alloxan has been used to induce DR in a large variety of animals including mice. Alloxan causes damage to pancreatic β cells and when given to mice 8–10 weeks of age can result in hyperglycemia following a single injection. Alloxan induces pericyte ghosts and loss of RGCs within 7 days and microaneurysms with increased acellular capillaries by 21 days in mice from the FOT_FB strain [157] and microglial changes, with thicker cell bodies and shorter dendrites by 3 months of age [123].

STZ administration is the most commonly used drug to initiate diabetes and causes disruption of pancreatic beta cells and leads to hyperglycemia. Hyperglycemia onset typically occurs within 2 weeks, regardless of dosage and can be maintained for up to 22 months [158]. DR phenotypes observed in STZ mice and rats [147,159] include increased

numbers of astrocytes and gliosis 4–5 weeks after onset of hyperglycemia [158], RGC loss at 6 weeks, permeability at 8 weeks (Figure 1) retinal inner nuclear layer (INL) and outer nuclear layer (ONL) thinning at 10 weeks and acellular capillaries and pericyte ghosts at 6–9 months (Figure 1) [160]. Additional changes such as increased mRNA expression of adhesion molecules have been observed as early as one week after the induction of diabetes [161,162].

Kern and Engerman first reported an animal model of DR induced by a galactose-heavy diet [163]. Mice developed hyperglycemia by 6 weeks of age. After 15 months of hyperglycemia, endothelial cell loss and increased acellular capillaries were observed. After 21 months, lesions including pericyte ghosts, microaneurysms, and retinal thickening were observed. While retinopathy takes longer to develop in these mice, the mice live longer than other models, allowing them to be observed over a longer period of time, up to 26 months [164]. Similarly, rats have been kept on high-galactose diets for over 2 years. However, allaxon, the STZ or galactose models do not result in retinal neovascularization.

4.2 Models of neovascularization

Typically, the “non diabetic” oxygen induced retinopathy (OIR) [165] is utilized to assess anti-angiogenic agents for potential translation to the clinics. The OIR model exploits that mice are born with only a partially developed vasculature and manipulation of oxygen levels post natally (postnatal days P7-P12) can influence vascular behavior, and as such are a model of retinal development. In normal mouse pups, formation of the deep vascular plexus (DVP) is initiated on P8; however, DVP development does not occur when pups are placed into high oxygen, but rather formation of the DVP is initiated several days after return to normal room air around P15. The classical endpoints that are evaluated are central vaso-obliteration and retinal neovascularization. Both vaso-obliteration and neovascularization can be evaluated on retinal flat mounts. Retinal crosssections are also used for quantitation of neovascularization using serial sections of whole eyes cut sagittally. Typically, between two and four sections on each side of the optic nerve, 30 to 90 nm apart are counted for neovascularization as described by Smith *et al* [165]. Vascular cell nuclei, identified under light microscopy with hematoxylin staining typically are considered to be associated with new vessels and if found on the vitreal side of the internal limiting membrane are counted. Thus the OIR model provides a quantitative measurement and may demonstrate that a drug is particularly robust if it shows a significant reduction in neovascularization when performed as a dose-response study.

4.3 Genetic models of DR

Genetic mouse models of DR include the *Ins2^{Akita}*, non-obese diabetic (NOD), *db/db* (*Lepr^{db}*), Kimba, and Akimba, which vary in mode of inheritance, disease etiology, pathology, and progression of disease. The *Ins2^{Akita}* mouse is a model for T1D that arises as a result of a missense mutation in the *Insulin 2* gene that leads to a conformational change in the insulin protein, causing the protein to accumulate in pancreatic β cells, leading to β -cell death. Diabetes onset is around 8 weeks with an increase in retinal vascular permeability and reactive gliosis. Disease progression continues up to 8 months of age, with a reduction in axons and dendrites of RGCs by 12 weeks, an increase in acellular capillaries at 36 weeks,

and an increase in leukocytes in the vascular wall, as a result of inflammation. Decreased numbers of cholinergic and dopaminergic amacrine cells are observed leading to a reduction in the thickness of the IPL and INL [166]. The *Ins2^{Akita}* mouse is useful for studying the early progression of DR and the neuroprotective effects of treatments, as loss of RGCs can be detected in a short span of time [167].

Another model of T1D is the NOD mouse that shows apoptosis of pericytes, endothelial cells, and RGCs, as well as retinal capillary basement membrane thickening starting as early as 4 weeks. Vasoconstriction and degeneration of major vessels with abnormal microvessels can be detected approximately 4 months after hyperglycemia. However, NOD mice exhibit a gender bias towards females becoming diabetic [168].

A well-established T2D is the db/db (*Lepr^{db}*) mouse that is deficient in the leptin receptor and spontaneously develops diabetes associated with obesity at 4–8 weeks of age. Six-month-old db/db mice have been shown to exhibit early features of DR, such as pericyte and endothelial cell loss, BM thickening [169] and increased blood flow in the retina. By 15 months, these mice demonstrated BRB breakdown, pericyte loss, neuroretinal apoptosis, glial reactivation, and acellular capillaries in the retina [170].

Similar to mouse models, there are several spontaneously diabetic rat models, the Zucker diabetic fatty rat, the WBN/KOb rat, the Otsuka Long-evans Tokushima fatty rat, the Goto-Kakizaki rat, the Torii rat [167] and the BBZ/Wor [171]. Each of these models recapitulates the major phenotypic characteristics of DR and are reviewed in detail in Robinson *et al* [167].

5. Age-related macular degeneration (AMD)

AMD is the leading cause of irreversible vision loss in individuals over 65 years of age. It is estimated that over 187 million people worldwide are affected by the disease [172,173]. It is characterized by a progressive and chronic degeneration of the macula, a 5.5 mm diameter region central in the retina, responsible for high acuity vision. Tissues and cells, which are affected and thus ‘vulnerable’ in AMD include, photoreceptors, RPE, Bruch’s membrane, and the outer blood supply of the eye including the choriocapillaries and choroidal endothelial cells [174]. Clinically, the pathogenesis of early dry AMD involves accumulation of lipid and protein rich extracellular deposits (e.g. sub-RPE deposits, basal laminar and linear deposits, drusen), below the RPE, the cells that support the overlying neural retina (Figure 2) [175–180]. As the disease progresses to the advanced stages it can manifest as either geographic atrophy/“late dry”, or exudative/“wet” AMD. Geographic atrophy is characterized by areas of RPE atrophy that can coalesce to become larger, along with loss of photoreceptor cells in the central macula, leading to vision loss [174,181]. Neovascular AMD is characterized by abnormal growth of choroidal blood vessels through Bruch’s membrane, generally confined below the RPE and/or retina, though it can also penetrate beyond the sub-retinal space and within the retina, progressing to becoming retinal angiomatous proliferation. These ‘new’ vessels are leaky and proliferative in nature and eventually may lead to fibrosis and scarring, contributing to significant vision loss [181,182]. Currently, there are no effective therapies available for treatment of early dry

AMD or geographic atrophy, and the anti-VEGF strategy commonly used in clinics to treat “wet” AMD has been shown to be effective in only a subset of patients [183]. This highlights the critical unmet need of developing more effective therapeutic strategies for the treatment of all clinical sub-types of AMD.

6. *In vitro* modeling of AMD

Cell-based modeling of chronic neurodegenerative diseases of aging such as AMD is challenging, as it requires patience and time. One concern often raised, is that the use of just one or even two cell types in a culture dish over simplifies pathogenic mechanisms that would occur in an otherwise complex tissue. However, this may in and of itself be an advantage as it allows for investigating the response of isolated cells under various conditions and challenges, which may reveal important insights into disease mechanisms. Furthermore, if standardized, cell cultures may serve as a platform for high throughput screening of pharmacological agents as potential therapies. RPE cells derived from human eyes have been used most frequently for modeling AMD phenotypic features and specifically with the goal of developing sub-RPE deposits such as drusen, the key pathology in early dry AMD, in a culture dish (Table 3). Recent successes include the culturing of fetal human RPE cells on to porous supports, which over time produce extracellular deposits containing apolipoprotein E (apoE) and complement proteins, molecules also identified in human drusen [184]. The presence of components of drusen including apoE, hydroxyapatite and neutral lipids have also been observed below porcine RPE cells cultured for up to 6 months on uncoated transwells [185]. Finally, patient-derived human-induced pluripotent stem cell RPE derived from patients with the dominant macular degenerations, Sorsby’s fundus dystrophy, Doyme honeycomb retinal dystrophy and malattia Leventinese, have been used as a resource for modeling sub-RPE deposit formation *in vitro* [186]. Of significance, cultures from patients with macular degenerations developed a greater number of deposits, with a different composition, when compared to normal aged individuals, highlighting the potential value of using these model systems to identify disease specific mechanisms that lead to drusen formation.

7. *In vivo* modeling of AMD

Animal models have proven to be invaluable in investigating the pathobiology of an aging disease as well as in testing of new therapies. An ideal model of AMD would be inexpensive, recreate the histological and functional changes, but progress in a rapid time course to allow for effective studies. Developing an efficient model of AMD that mimics both the early and late characteristics of AMD has been challenging due to several hurdles. First, development of AMD is a complex process, which involves genetic and environmental factors. Not only have numerous genetic polymorphisms and loci been reported to contribute to increased risk for AMD, but multiple biological pathways have also been shown to be dysregulated in the pathogenesis of AMD [175]. These include but are not limited to, oxidative stress, inflammation, angiogenesis, lipid and carbohydrate metabolism. Second, is the issue of anatomical differences between the species, in which the models have been developed, and the human retina. Despite these obstacles, models of AMD have been created in mice, rats, rabbits, pigs and non-human primates, which collectively have led to the

discovery of multiple facets of the pathology of the disease [187–193]. Rodents are advantageous due to their relatively short life-span, low cost and rapid disease progression. Importantly, they are amenable to genetic manipulation. However, as mentioned, both mice and rats lack a distinct anatomical macula and fovea, unlike humans in which the characterizing features of the disease are defined as occurring in the cone-rich macula. Non-human primates, on the other hand, form the other end of spectrum. They are closest to humans in terms of the anatomy of the macula but are expensive to maintain, difficult to manipulate genetically, and have a slow disease progression.

In general, the goal in developing an animal model of AMD is to recreate classical histological features, which have been reported from the eyes of patients with AMD. These include sub-RPE basal laminar deposits and basal linear deposits, and drusen; thickening of Bruch's membrane, RPE atrophy, accumulation of immune cells in the inner and outer retina, photoreceptor atrophy, choriocapillary atrophy, choroidal neovascularization and fibrosis (**Figure 2 and 3**) [194]. Decrease in functional activity as measured by electroretinography (ERG), is also observed in AMD patients and a desired outcome [195]. We will review several select animal models, which were developed following manipulation of genetic or environmental factors impacting reported AMD pathogenic pathways, and highlight the disease relevant pathology they exhibit (Table 4).

Oxidative injury

The retina is susceptible to oxidative damage due to its high metabolic demand, high concentration of oxidation prone polyunsaturated fatty acids and photosensitive molecules such as rhodopsin and lipofuscin, which can produce reactive oxygen species when exposed to light [196]. Oxidative damage from sunlight or cigarette smoking has been shown to be AMD risk factors in several epidemiological studies [197–199]. These observations have been the impetus to the development of animal models by using mice that lack intrinsic anti-oxidant mechanisms or exposing animals to various oxidant insults.

Super oxide dismutase (SOD) is a powerful anti-oxidant and is expressed in three forms, SOD1, SOD2 and SOD3. SOD1, is primarily cytosolic and is highly expressed in retina, SOD2 and SOD3, localize primarily to the mitochondria and extracellular space, respectively [200]. *Sod1*^{-/-} mice, after 7 months of age display 'drusen'-like deposits between the RPE and Bruch's membrane. These deposits express biomarkers of drusen including: vitronectin, carboxymethyl lysine and tissue inhibitor metalloproteinase 3 [201,202]. Light exposure in these mice can accelerate the appearance of 'drusen'-like deposits in a dose dependent manner. Interestingly, 10% of aged *Sod1*^{-/-} mice display evidence of spontaneous CNV by histology and fluorescein imaging, making this model one of the few to date to develop features of both dry and wet AMD. *Sod1*^{-/-} mice also exhibit progressive visual decline, reflected by a decrease in their a- and b- wave amplitudes and corroborated by an increase in the occurrence of necrotic cell death in the INL as compared to wild-type mice [203]. On the other hand, *Sod2*^{-/-} mice, die soon after birth due to dilated cardiomyopathy, which limits their use as a model. This is unfortunate as polymorphisms in *Sod2* gene have been associated with increased risk of AMD development [204,205]. To counter this, mice in which their RPE and retina have been transfected with an adenovirus

expressing a ribozyme capable of degrading Sod2, have been produced. These mice exhibit increased expression of markers of oxidative damage, thickening of Bruch's membrane, RPE degeneration, increased autofluorescence and photoreceptor atrophy, all characteristic features of dry AMD [206].

Cigarette smoking, second to aging, is a significant preventable environmental risk factor for the development of AMD as cigarette smoke contains over 5000 potential harmful compounds including many pro-oxidants. The role of cigarette smoke to induce or exacerbate AMD has been investigated in several animal models, both in isolation as well as in combination with a high fat diet and blue light exposure [207–210]. In these models, it has been demonstrated that when mice are stressed with oxidants including cigarette smoke, blue light, and/or high fat diet, they develop a thickened Bruch's membrane and basal laminar deposits, making them good models for investigating either how oxidants contribute to basal deposit formation or testing therapies to target deposit formation. The potential role of cigarette smoke and xenobiotic metabolism in AMD pathology has further been corroborated by investigating the phenotype of the aryl hydrocarbon receptor (AhR) knock out mice (*Ahr*^{-/-}). AhR is a transcription factor responsible for xenobiotic metabolism and detoxification and can be activated by components of cigarette smoke. Furthermore, AhR activity in human RPE cells decreases with increasing age, suggesting a decline in the ability of RPE cells to effectively clear out toxins through this pathway [211]. *Ahr*^{-/-} mice exhibit decreased visual function and develop several phenotypic features of dry AMD including disrupted RPE cell junctions, lipid- and collagen-rich basal deposits, Bruch's membrane thickening and progressive choroidal atrophy (Figure 4) [211,212]. Interestingly, aged *Ahr*^{-/-} mice are also susceptible to developing large and complex neovascular lesions following laser injury in comparison to age-matched wild-type mice [213]. These studies have revealed a novel pathway contributing to development of AMD pathology and support investigating the role of AhR agonists and specifically acute activation of the AhR as a potential therapeutic strategy for early and dry AMD.

Lipid transport and metabolism

Several parallels have been drawn between the deposition of cholesterol and lipids in atherosclerotic plaques and the deposits that accumulate under the RPE and within Bruch's membrane [173,214–216]. Lipids and cholesterol accumulate within Bruch's membrane with aging and their hydrophobic nature may interfere with the conventional passage of metabolites between the RPE and the outer retinal blood supply [216–218]. They have also been reported to contribute to accumulation of basal laminar and basal linear deposits [219]. Finally, a higher consumption of cholesterol, monosaturated, polyunsaturated and saturated fats; and genetic polymorphisms in apolipoproteins, which regulate lipid transport, have also been shown to be risks associated with AMD development [220–223]. These observations have served as the inspiration for the creation of various animal models, which explore the relationship between lipid transport dysregulation and AMD.

Basal laminar deposit accumulation has been demonstrated to occur in old (16 months) C57BL/6 mice fed high-fat diets [207]. In combination with an additional stressor such as exposure to blue-green light and/or estrogen depletion, the number and severity of deposits

has been shown to increase, supporting the role of diet as a risk for AMD development [207,224]. Apolipoprotein molecules, which mediate lipid and cholesterol transport, are found in drusen and as such, the retinal morphology of apolipoprotein knockout mice have been examined extensively. For example, *apoE* deficient mice develop high levels of circulating cholesterol [225] and histological evaluation of the retinas of 8 month old *ApoE*^{-/-} mice has revealed they develop a thicker Bruch's membrane containing membranous material when compared to age-matched controls [226]. The retinal morphology of transgenic animals where the mouse *ApoE* was replaced with different human APOE alleles has also shed light on their role in AMD pathology development [227–229]. For example, aged *APOE4* targeted replacement mice, when fed a high fat diet over 8 months, develop Bruch's membrane thickening, RPE pigmentary changes, and sub-RPE deposits. A small percentage of these aged *APOE4* mice also displayed areas of CNV and photoreceptor loss. Another apolipoprotein found in abundance in drusen is apolipoprotein B100, a major component of low-density lipoprotein (LDL) cholesterol-carriers [230]. Transgenic mice expressing human *APOB100*, when fed a high fat diet and exposed to oxidative blue-green light have been shown to develop basal laminar deposits [231], a phenotype that is exacerbated with age resulting in loss of RPE basal infoldings, basal laminar and linear deposits and Bruch's membrane thickening [232,233].

Peroxisome proliferator-activated receptor β/δ (PPAR β/δ), is a ligand-activated transcription factor, which is involved in the regulation of lipid metabolism, extracellular matrix remodeling and inflammation, pathways also associated with AMD development. Aged *PPAR β/δ* ^{-/-} mice have been shown to develop several features of dry AMD including, Bruch's membrane thickening, discontinuous sub-RPE deposits, loss of RPE basal infoldings, RPE degenerative changes and lipofuscin accumulation when compared to age matched wild-type mice [234]. Interestingly, absence of the PPAR β/δ resulted in less severe choroidal neovascular lesions forming following laser injury, in comparison to mice expressing the receptor. Importantly, treatment with PPAR β/δ antagonist demonstrated its therapeutic potential in the experimental mouse model of CNV [234]. Collectively, these studies highlight the importance of studying regulators of lipid metabolism in the pathobiology of AMD. Furthermore, the models discussed are suitable for further in-depth investigation of potential mechanisms contributing to- and/or development of candidate therapeutic strategies that specifically target RPE degenerative changes, sub-RPE development or CNV formation, with consideration of a patient's dietary habits or systemic lipid levels.

Immune dysregulation

Several genome-wide association studies have established a role of immune system dysregulation in AMD progression. These studies revealed an association between single nucleotide polymorphisms (SNPs) encoding components of the immune system and increased risk for developing advanced AMD [235–239]. Additionally, multiple histological studies examining human donor tissue have identified various components of the immune system within drusen, the choriocapillaries and choroid [240].

Complement factor H (CFH) was the first complement gene associated with increased risk of developing AMD, with polymorphisms including Y402H, increasing the risk of advanced disease development [236–239]. Additionally, inheritance of Y402H has been reported to confer resistance to anti-VEGF and thus has potentially negative implications for treatment outcomes in patients with CNV [241,242]. CFH acts to regulate the alternative complement pathway and several CFH-based mouse models have been generated to study its impact on AMD pathology. *Cfh*^{-/-} mice display retinal dysfunction, RPE autofluorescence and progressive C3 deposition [243,244]. By contrast, *Cfh*^{Y402H} transgenic mice, which express the human Y402H variant in the RPE and liver, driven by the apoE promoter, exhibit basal laminar deposits and accumulation of lipofuscin-like material in the RPE [245].

Interestingly, this phenotype is accompanied by accumulation of macrophages and immune cells in the outer retina. Additionally, comparison of pathology in aged *Cfh*^{-/-} versus *Cfh*^{+/-} mice when challenged with a high fat diet, has revealed greater RPE damage and visual function impairment in mice with decreased levels of *Cfh* rather than complete absence of the gene [246]. In a follow-up study it has been shown that mice that express the full length human CFH when crossed with *Cfh*^{-/-} mice demonstrate improved visual function, supporting the therapeutic potential of increasing CFH levels [247]. Similarly, low levels of CFH have been associated with development of a more severe laser induced CNV lesion in mice [248,249]. Collectively, these studies have begun to shed light on the complexity of the complement pathway in the development of early dry AMD. It is important to note, that alterations in CFH levels have not resulted in spontaneous expression of features of either GA or CNV in mice, perhaps reflecting differences in immune regulation between mice and humans.

Chemokines or their receptors have also been associated with an increased risk of AMD. Monocyte chemoattractant protein 1, also known as CCL2 and its receptor CCR2 are involved in recruitment of immune cells at the sites of injury, and CCL2 levels are elevated in the aqueous humor, and urine of AMD patients [250]. Additionally, loss of function in CX3CR1 due to T280M mutation has been implicated in the development of AMD [251]. However, to date, chemokine knockout mouse models have failed to convincingly demonstrate primary AMD pathology such as drusen, RPE atrophy and/or spontaneous CNV. For example, *Cx3cr1*^{-/-} display hypertrophic microglia in the sub-retinal space. It has been hypothesized that loss of *Cx3cr1* function results in death of photoreceptors, which are then phagocytosed, contributing to drusen formation. Tuo *et al* developed an accelerated model of AMD by crossing *Cx3cr1*^{-/-} and *Ccl2*^{-/-} mice, which developed thickening of Bruch's membrane, RPE changes, photoreceptor atrophy and neovascularization extending into the retina, in as early as 6 weeks of age [252]. It was later discovered that these findings may have been complicated by the retinal expression of the *Crb*^{rd8} mutation [253]. Beyond chemokines, there are several other models in which immune cell recruitment into the sub-retinal space has been reported, following laser induced experimental CNV, including in aged *Ahr*^{-/-} and *PPARβ/δ*^{-/-} mice [213,234]. Collectively, these studies support a contributing role of chemokines and the involvement of immune cells in the development of AMD and highlight some of the models that may be used to test therapies targeting accumulation of immune cells below the retina or within CNV lesions.

8. Conclusions

The challenges we continue to face in understanding the pathogenesis of complex retinal diseases such as DR and AMD, are dependent on a better appreciation of the various nuances of human pathology and attempting to recreate these features in *in vitro* and *in vivo* models. This may be in part dependent on better characterization of disease phenotypes in association with patient genotypes. The *in vivo* models that have been produced so far, take advantage of the risk factors that have been identified to date and each develop some characteristics of disease, but not all of them. As such they are often criticized as not being the ‘perfect’ model such that the search continues. Yet it remains to be seen whether or not developing a ‘perfect’ model of a complex retinal disease such as DR or AMD, though meritorious, would be an achievable goal, given the clinical and epidemiological information we have so far, which indicate that there are no patients that express all risk factors identified for the disease nor are there patients that present with all the clinical phenotypic features. It is arguable that the quest for the ‘perfect’ model of DR and AMD until better phenotypic-genotypic associations and sub-classifications of each of these complex diseases are available, may be a futile one. Nevertheless, this does not diminish the value of the models currently available, each of which are credible resources and given an appreciation of the disease relevant pathology they exhibit, may be used to not only identify new signaling pathways contributing to initiation and progression of disease but also new therapeutic targets to pursue. Given the availability of possible drugs for therapeutic testing, these models would be valid platforms to investigate drug efficacy, in a targeted manner, and on specific pathological features. Thus it is critical during experimental design to be aware of the disease relevant endpoints and utilize the appropriate model that will facilitate an accurate interpretation of the therapeutic potential of a drug on disease outcome.

9. Expert opinion

As mentioned earlier, the multi-factorial nature of complex non-Mendelian retinal diseases such as DR and AMD has made understanding the pathogenesis and identification of effective therapeutic treatments challenging. However, as highlighted in our review, there are a number of useful *in vitro* and *in vivo* tools that are highly relevant and have already led to breakthroughs and clinically approved therapies. Critical to this goal, has been identification of the appropriate model to use in order to test a potential therapy, identifying the appropriate endpoint by using the model, as well as appreciating possible drawbacks of the model. Currently, the use of a specific model to target one particular aspect of the disease in question has garnered considerable popularity; however it may also add cost, should additional aspects of the disease and thus additional experimental models need to be investigated. Validation of pharmacological agents follows this same logic necessitating the selection of an *in vivo* model that exhibits the pathology being addressed by the drug. Similarly, *in vitro* models can mimic features of a disease and allow for extensive dose-response and time-course studies to be performed efficiently and in a cost effective manner. For DR, the combination of type 1 and 2 diabetes animal models that progress to non-proliferative stages of disease as well as the OIR animal model, which features neovascularization during development, rather than associated with diabetes, has already promoted significant advances in our understanding of DR pathogenesis and led to recent

success of steroids and anti-VEGF treatments. Analogously, the most popular model used to identify potential therapies for the wet clinical sub-type of AMD has been the laser induced choroidal neovascularization mouse model and though it diverges in some respects from the human form, pre-clinical studies with this model led to clinical studies that eventually approved anti-VEGF therapies for treatment of neovascular AMD. This model continues to serve as the 'go to' model to test emerging new therapies.

It is important to note, that though the etiology of DR and AMD are quite different they share common pathogenic mechanisms associated with their initiation and progression. Additionally, there is overlap in the number of cells that are affected in both diseases. The RPE cells serve a good example, in that they constitute a common denominator in the disease pathology of both DR and AMD, yet are characterized by their ability to regulate the cytokine milieu in the outer retina, their interactions with overlying photoreceptors, as well as the underlying Bruch's membrane and choroidal vasculature. With this in mind, should a therapy be identified that could strengthen ailing RPE cells, it would be useful to consider its potential for both diseases. Likewise, improving aberrant endothelial cells (retinal in origin in the case of DR and choroidal in the case of AMD) offer a common target for treating the neovascular components of both retinal diseases. The use of anti-VEGF approaches to treat neovascularization in both DR and AMD presents the text book example of taking advantage of the common pathogenic pathways involved in two different retinal diseases. After the identification of VEGF as an angiogenic molecule, anti-VEGF therapy was first shown to be effective in inhibiting retinal neovascularization in models of retinal ischemia and retinopathy of prematurity, which was subsequently tested as a treatment for choroidal neovascularization associated with advanced AMD. Additional examples of drugs currently under investigation for both DR and AMD include targeting various growth factors as well as components of the complement pathway. Thus, it is safe to say that future studies and breakthroughs in identifying potential therapies for complex diseases will benefit from an awareness of common pathways and cell-types affected, as they may present new avenues for drug discovery including repurposing existing drugs for new indications.

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List of abbreviations:

AMD	Age-related Macular Degeneration
DR	Diabetic Retinopathy
RPE	Retinal Pigment Epithelium
REC	Retinal Endothelial Cells

BREC	Bovine Retinal Endothelial Cells
HREC	Human Retinal Endothelial Cells
PECAM	Platelet Endothelial Cell Adhesion Molecule
ERG	Electroretinography
VEGF	Vascular Endothelial Growth Factor
ICAM-1	Intercellular Adhesion Molecule-1
VCAM-1	Vascular Cell Adhesion Molecule-1
LDL	Low Density Lipoproteins
PEDF	Pigment Epithelial-Derived Factor
CACs	Circulating Angiogenic Cells
RGSs	Retinal Ganglion Cells
STZ	Streptozotocin
INL	Inner Nuclear Layer
ONL	Outer Nuclear Layer
IPL	Inner Plexiform Layer
NOD	Non-Obese Diabetic
BRB	Blood Retina Barrier
SOD	Superoxide Dismutase
CNV	Choroidal NeoVascularization
AhR	Aryl Hydrocarbon Receptor
Apo	Apolipoprotein
PPAR	Peroxisome Proliferator Activated Receptor
CFH:	Complement Factor H
GA	Geographic Atrophy
SNP	Single Nucleotide Polymorphism
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
OIR	Oxygen Induced Retinopathy
DVP	Deep Vascular Plexus

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Article highlights box

- Current treatments for complex retinal diseases including diabetic retinopathy (DR) and age-related macular degeneration (AMD), major causes of vision loss in the young and elderly populations, are limited.
- *In vitro* and *in vivo* models are valuable tools to not only study the pathogenesis of disease, but also identify new therapeutic targets.
- The complexity of retinal diseases such as DR and AMD, may preclude development of the ‘perfect’ model, which features all the phenotypic and genotypic characteristics of the human disease.
- Rational design should be employed when using models currently available in pre-clinical studies to validate the efficacy of drugs; cognizant of the strengths and weaknesses of the model, as well as the phenotype or pathogenic pathway being targeted.
- New avenues for drug discovery may arise from breakthroughs in identifying potential therapies for other complex diseases, with which DR and AMD share common pathways and/or cell-types affected.

Figure 1. Histology of retinal vascular permeability and acellular capillaries in diabetic rodents. Vascular permeability in (A) wild-type control versus (B) STZ-induced diabetic mouse after 8 weeks of diabetes. Acellular capillaries are rare in control C57BL mice (C) or Wistar rats (E), but evident 9 months after STZ induction of diabetes (D-red arrows) or 7 days after ischemia-reperfusion (F-black arrows). *This panel includes original images.*

Figure 2. Pathology of sub-RPE deposits in human AMD samples.

Transmission electron microscopy images reveal details of the complexity of sub-RPE deposits including the presence of basal lamina deposits (A-red double arrowhead), fibrous long spacing collagen (B-red arrow), and membranous debris (C-green double arrowhead). *This panel includes original images.*

Figure 3. Abundance of immune cells in human AMD.

Cryosections from human donor eyes were probed with antibodies to Iba1, which stains microglia and macrophages (green), SV2, a synaptic vesicle marker (red) and Hoechst, which highlights cell nuclei (blue). Iba1 positive cells are absent from the retina of an aged donor without eye pathology (A), but present in the retina of a patient with wet AMD both in regions where CNV is absent (B) and present (C). Furthermore, an abundance of Iba1 positive cells are seen throughout the CNV lesion itself (C-CNV lesion boarder is indicated by yellow broken line). *This panel includes original images.*

Figure 4. Pathology of sub-RPE deposits in a mouse model.

Aged *AhR* knockout mice develop clinical phenotypic features of dry AMD including accumulation of neutral lipids within sub-RPE deposits as evident by staining of cryosections with oil red o (A-wildtype, B and C-*AhR* knockouts). Transmission electron microscopy reveals that while wild-type mice do not develop sub-RPE deposits (D), thick and continuous deposits occur frequently in *AhR* knockout mice (E, F-red double arrowhead). *This panel includes original images*

Table 1.*In vitro* models used to study DR.

Assay	Cell type	Read-out
<i>Permeability</i>	BREC RPE HREC-pericyte co-culture	Monolayer permeability in response to VEGF and cytokines [8,74,77,147] Tight junctions structure and function[35,84,85]
<i>Pro-inflammatory changes</i>	HREC BREC Müller cells Pericytes HREC-pericyte co-culture HREC-Müller cell co-culture RPE Photoreceptors	Cytokines and adhesion molecule expression and leukocyte adhesion in response to diabetogenic media: • lipids[30,36,42,72,90] • glucose [12,14,27,32,35,47,65,89–94] • cytokines[27,33,36,43,72,73,84–89] High glucose-induced, oxidized glycated LDL and cytokine-induced cytokine and growth factor production, ER stress[102–107]. Response of HREC (adhesion molecules etc.) to pericyte and Müller cell-produced cytokines High glucose and aldose reductase activation and hypoxia-induced cytokine and growth factor production[128–135] Hypoglycemia-induced proinflammatory changes[146]
<i>Apoptosis</i>	HREC BREC Pericytes RPE	Diabetogenic media-induced apoptosis [13,20,28,92,97–101]
<i>Tube formation</i>	HREC HREC and pericyte co-culture HREC and CACs co-culture	Tube length, width and branching points under control, pro- and anti-angiogenic and diabetogenic conditions[36,254]

Table 2.

Animal models that exhibit DR pathology.

DR Pathology	Animal Model
Acellular capillaries	STZ induced T1D [158] db/db [169,170] Galactose [163]
Pericyte ghosts [149]	STZ induced T1D [158] db/db [169,170] Galactose [163] Alloxan [157]
Microglial activation	STZ induced T1D [158] Alloxan [157]
Loss of RGCs, INL and ONL thinning	STZ induced T1D [158] Ins2Akita[166,167] Alloxan [157]
Microaneurysms	Galactose fed mice
Astrocytes/gliosis	STZ; galactose [163] Ins2Akita [166]
Permeability	STZ induced T1D [158] db/db [169,170] Galactose[163] Ins2Akita [167]
Leukocytosis	STZ induced T1D [158] db/db [169,170] Galactose [163] Ins2Akita [167]
Capillary basement thickening	NOD mice [167] STZ induced T1D [158]

RGC=retinal ganglion cells; INL=inner nuclear layer; ONL=outer nuclear layer; STZ=streptozotocin; NOD=non-obese diabetic; T1D=type 1 diabe

Table 3.*In vitro* models used to study AMD.

<i>In vitro</i> models	Advantages	Disadvantages
Human RPE from adult eyes	<ul style="list-style-type: none"> • Easy to isolate and culture from post mortem eyes. • Can be cryopreserved and cultured. • Can be used to study molecular pathways implicated in AMD, e.g., lipid transport, metabolism, etc. • Can be used to study the effect of various insults, e.g cigarette smoke, oxidized lipids, etc. • Amenable to transfection (siRNA and plasmid) 	<ul style="list-style-type: none"> • Lacks tissue complexity; e.g. choroid and photoreceptors. • Lacks immune system components. • Sub-RPE deposits and known constituents of drusen are generally absent. • Loss of RPE characteristics with passage.
Human RPE from fetal eyes	<ul style="list-style-type: none"> • Easy to grow on porous support. • Can be cryopreserved and cultured. • Form sub-RPE structures which stain positive for known drusen components including apoE, vitronectin, clusterin, amyloid, etc. • Can be used to study the role of component system as apoE positive deposits co-localize with component system molecules in the presence of serum in growth conditions. • Good platform to test the effect of drugs on deposit formation as well as complement activation. 	<ul style="list-style-type: none"> • Lacks tissue complexity; e.g. choroid and photoreceptors. • It takes about 1-3 months for deposit formation.
iPSC-RPE derived from human	<ul style="list-style-type: none"> • Provides a suitable platform to investigate the RPE-autonomous molecular events. • Can be used to study patient specific effects like age and genetics (point mutations or polymorphisms) on drusen formation and response to therapy. • Conducive to genome editing by CRISPR-Cas9 system. • Starting material is convenient to obtain, e.g. skin biopsies, blood. 	<ul style="list-style-type: none"> • Lacks tissue complexity; e.g. choroid and photoreceptors. • Reprogramming is an expensive and time-consuming process. • Extensive characterization is required at each step of dedifferentiation and differentiation.
Porcine RPE cells	<ul style="list-style-type: none"> • More readily available than human post mortem eyes. • Cultures produce focal and diffuse sub-RPE deposits similar to the ones seen in human AMD macula. • Can be used to test the effect of drugs on deposit formation 	<ul style="list-style-type: none"> • Lacks tissue complexity; e.g. choroid and photoreceptors. • Long culture time

Table 4.

Testing of potential therapies on various AMD pathologies in mice.

Models of AMD Pathologies	Candidate drugs	Effect on AMD pathogenesis	References
Oxidative stress			
<i>Sod</i> ^{-/-}	α -Lipoic acid	Increase in serum SOD	[255]
<i>AhR</i> ^{-/-}	AhR agonists	Ameliorate CNV formation	Unpublished
Immune regulation			
<i>Ccl2/Cx3cr1</i> ^{-/-}	Nutrient supplementation with lutein and zeaxanthin Naloxone	Lesion regression Lower VEGF, TNF α , iNos, Cox2 expression in retina Lower number of microglia in outer retina	[256] [257]
<i>Ccr2</i> ^{-/-}	TNF α -stimulated gene/protein (TSG-6)	Lowered VEGF expression Lower infiltration of CCR2 ⁺ cells in CNV lesions	[258]
Complement pathway	IBI302 Eculizumab Anti-factor D (lampalizumab)	Novel bispecific decoy receptor fusion protein with a VEGF inhibition domain and a complement cascade inhibition domain Binds C3b/C4b Anti-mouse complement component 5 antibody Inhibit CNV Inhibit infiltration of F4/80-positive cells into CNV lesions Humanized IgG Fab fragment directed against factor D Inhibit systemic Alternative Complement activity	[259] [260] [261]
Lipid transport and metabolism			
<i>Pparβ/δ</i> ^{-/-}	GSK0660	Ameliorate CNV formation Regulate extracellular matrix deposition in CNV lesions	[234]

AhR=aryl hydrocarbon receptor; SOD=superoxide dismutase; CNV=choroidal neovascularization; VEGF=vascular endothelial growth factor; TNF α =tumor necrosis factor alpha; iNos=inducible nitric oxide synthase; Cox2=cytochrome C oxidase; Ccl2=CC chemokine ligand 2; Ccr2=cc chemokine receptor; PPAR=peroxisome proliferator activated receptor.