membrane trafficking and cellular morphogenesis. OPTN is induced by TNF-α and binds to an inhibitor of TNF-α and the adenovirus E3-14.7 kDa protein. To determine the effects of human glaucoma mutations in a transgenic mouse system, mice over-expressing wild type OPTN, OPTN carrying the glaucoma associated mutation E50K, and OPTN with exon5 deleted were constructed. Although wild-type OPTN do not show any abnormalities and the exon 5 deleted construction was found to be lethal prenatally, mice transgenic for the E50K mutant OPTN show steep optic nerve cupping with rearrangement of supporting tissue and blood vessels 18 weeks after birth (see Figure 68.6). The RGC and astrocyte loss observed is similar to the end phase changes seen in human glaucoma patients. Understanding the mechanism underlying normal tension glaucoma in these transgenic mice will enhance our understanding of each step leading to optic nerve cupping and how to prevent it. Based on the success of the mouse model, use of larger animals such as transgenic rabbits or pigs, in which more precise measurement of IOP and trials of surgical procedures suitable for therapy in humans are possible are currently being investigated.

Other glaucoma mouse models have been made through genetic manipulation. Knockout and transgenic mouse models of myocilin were made to answer the question whether elevated expression of the myocilin protein can influence the IOP (Gould *et al.*, 2004). Up to a fifteen-fold increase in myocilin expression failed to result in elevation of the IOP, any abnormality of retinal ganglion cells, or cupping of the optic nerve head. Mice lacking the cytochrome P450 1B1 (CYP1B1) gene were generated on B6 and 129X1/SvJ mouse stains (Libby *et al.*, 2003). Both strains were affected by the CYP1B1 deficiency with focal angle abnormalities, but 129X1/SvJ albino strains lacking tyrosinase were more severely affected, suggesting the presence of tyrosinase as an important developmental molecule.

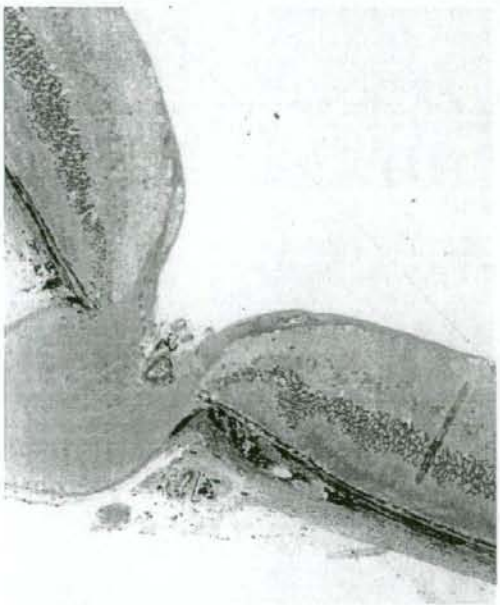

Figure 68.6 Histological section demonstrating excavation of the optic disc in an 18-week-old E50K mutant OPTN transgenic mouse.

Conclusion

In this chapter we have provided a brief overview of age-related eye diseases and the current state of knowledge and research on three of these. Age-related cataracts, age-related macular degeneration, and progressive open angle glaucoma account for much of the population burden imposed by age-related eye diseases. Although no perfect system to study these diseases exists today, an increasing number of experimental models are being developed. Although none is an exact replica of the clinical disease and should not be applied indiscriminately, each of these can provide useful information on some aspects of the disease in humans. They promise to accelerate the pace of research and provide mechanistic and therapeutic insights into the diseases that threaten sight in our aging population.

Recommended Resources

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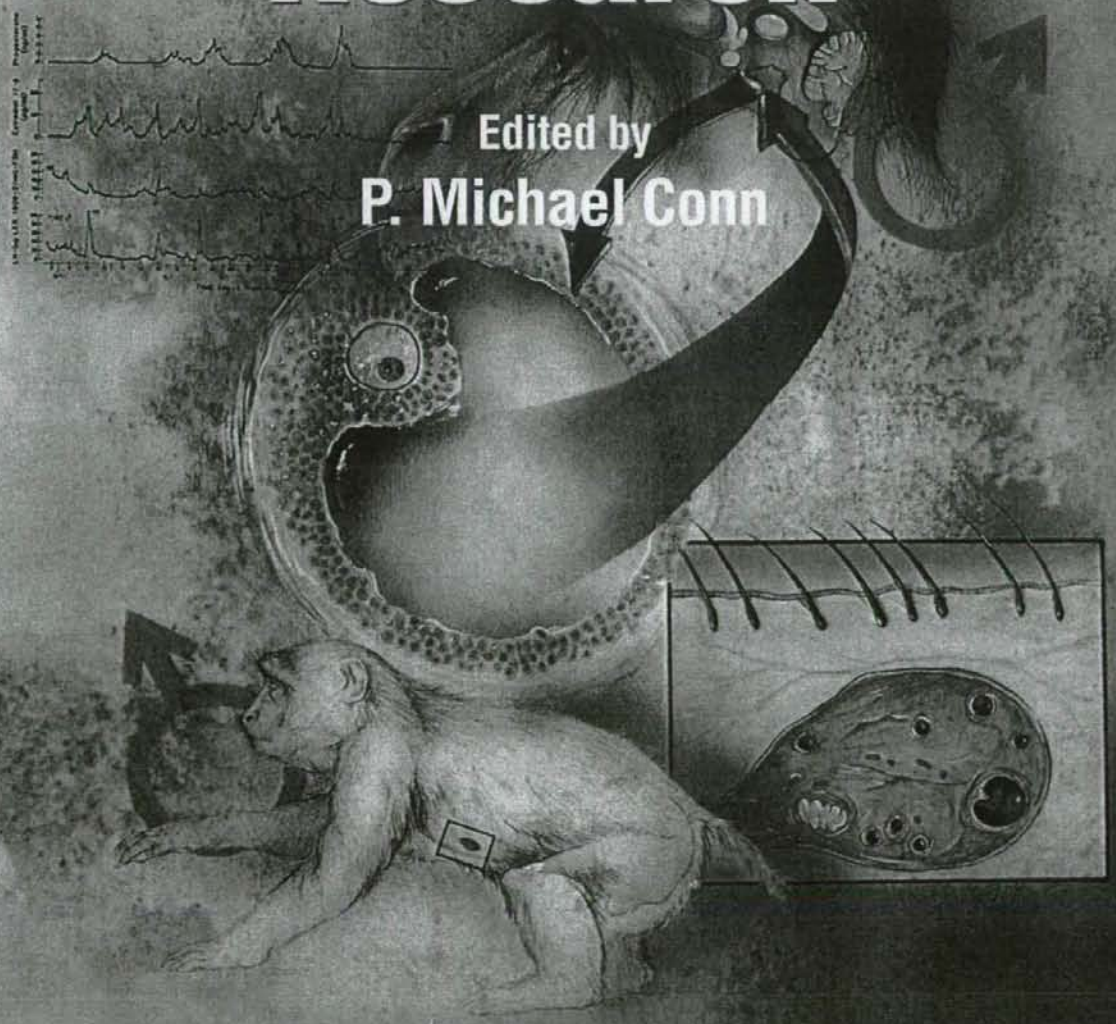
Source Book of Models for Biomedical Research



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Source Book of Models for Biomedical Research

Chapter 33: Animal Models for Eye Diseases and Therapeutics

Subtitle: Animal models of Age-Related Macula Degeneration and glaucoma

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***Key Words**: Vision, Age-related macular degeneration, Retina, Macula, Drusen, Glaucoma, Retinal ganglion cells, Optic nerve*

1. Visual impairment and importance of animal models for eye diseases

It is believed that more than 80% of the information our brain receives comes from the visual system. Dysfunction of the visual system can alter the normal human life style and significantly lower the quality of life. The causes of visual impairments and blindness vary among ethnic groups and the global regions where they live. There are many causes of visual impairments including diabetic complications, infections, and trauma, however the most prevalent causes of visual impairment are cataracts, glaucoma, and age-related macular degeneration (AMD). According to the World Health Organization, there were more than 161 million visually impaired individuals in 2002, and 124 million of this group had low vision and 37 million were blind (http://www.who.int/mediacentre/factsheets/fs282/en/index.html) (Fig. 1).

Cataract, glaucoma, and AMD are responsible for 69% of blindness globally. Although cataracts are the leading cause of blindness worldwide, recent advances in cataract surgery has significantly reduced the visual impairments caused by cataracts especially in developed countries. Glaucoma, an optic neuropathy, is often associated with elevated intraocular pressure and is responsible for blindness in 6.7 million people

across the world. Glaucoma is more common in individuals of African ancestry, and the incidence of glaucoma increases with age.

The most prevalent eye disease for elderly Europeans and Americans is AMD. This degenerative disease progresses from retinal deposits called drusen to neovascularization and retinal hemorrhages resulting in irreversible loss of central vision.

In spite of the high incidence of AMD and glaucoma, a limited amount of information is available on the underlying pathological mechanisms causing these diseases. Obtaining tissues for any disease is often difficult, and even when obtained, they may not be informative because the tissues are usually collected many hours or even days after death. Because experimental studies of AMD and glaucoma are limited in humans, the availability of animal models is very valuable because they can be used to investigate the molecular mechanisms causing these diseases and to test new therapeutic interventions. Animal models, compared to other experimental methods, e.g., cell and organ cultures or postmortem models, allow the study of different pathological factors and therapeutical treatments under in vivo conditions, i.e., with the visual and other systems of the body intact. Appropriate animal models, e.g., monkey,

mouse, zebrafish, also facilitate the identification of new genes involved in the pathology as well as elucidate the genetic relationships between causative and modifier genes. Equally important, specific genes can be altered in these models. Thus, it is possible to induce mutations in animals, and then search for specific phenotypes, e.g., abnormal intraocular pressure (IOP) and retinal ganglion cell degeneration. Subsequently, the affected genes can be identified by standard genetic procedures.

Many animal models of AMD and glaucoma have been developed in different mammalian and non-mammalian species. None of these models is a perfect reproduction of the human disease, and when choosing the animal model for research, the investigator should evaluate the following: 1) similarity of the visual system of the model to that of humans especially the eye; 2) similarity of the time course of pathological changes in the model and in human eyes; 3) ability to perform genetic manipulations; 4) training required to produce affected animals; 5) size of the eye; 6) availability and difficulties in the methods of analysis; 7) availability of animals; and 8) cost.

2. Age-related macular degeneration (AMD)

2.1. Introduction of AMD

The retina is composed of nine layers of neural and glial cells that are arranged concentrically at the posterior pole of the eye. Incoming light is focused on the central area of the retina called the fovea which is located in the center of the macular area (Fig. 2). In humans, the size of the macula is approximately 6 mm in diameter (Fig.3). The outer (posterior) surface of the retina is covered by a monolayer of retinal pigment epithelial (RPE) cells which forms a diffusion barrier between the neural retina and the choroidal blood supply. The RPE regulates the transport of proteins to the retina, and controls the hydration and ionic composition of the subretinal space. The physiological condition of the RPE is closely associated with the pathogenesis of AMD.

AMD is a blinding disorder characterized by a marked decrease in central vision associated with RPE atrophy with or without choroidal neovascularization (CNV). Many factors including genetic, behavioral, and environmental, are involved in this disease. AMD is characterized by the degeneration of cone photoreceptors in the foveal region of the retina resulting in a decrease of central visual acuity. The progressive impairment of

the retinal pigment epithelial (RPE) cells, and damage to Bruch's membrane and choriocapillaris results in retinal atrophy and photoreceptor dysfunction. In some cases, CNV develops, and the new vessels penetrate Bruch's membrane and pass into the subretinal space.

Two types of AMD are recognized; the non-neovascular type is called the dry-type AMD and includes more than 80% of the cases, and the neovascular type is called the wet-type AMD which is progressive with a higher probability of blindness. The prevalence of AMD differs considerably among the different ethnic groups, but the incidence increases with age in all groups. A lower prevalence of AMD has been reported in individuals of African ancestry than of Anglo-Saxon ancestry. Other risk factors for AMD are cigarette smoking, obesity, hypertension, and atherosclerosis.

2.2. Epidemiology and genetics of AMD

Extensive epidemiological studies have shown a genetic component for AMD. Thus, twin studies have shown a higher concordance for AMD in monozygotic twins than in dizygotic twins (1-3). In addition, first degree relatives of individuals with AMD

have a 2 to 4 fold higher incidence of AMD over individuals without a family history of AMD. Genetic segregation studies have also shown a genetic effect that accounts for approximately 60% of AMD with a single major gene accounting for about 55% of the risk of developing AMD. Overall, the data have suggested that the etiology of AMD has a significant genetic component. Only a small proportion of the families with AMD show Mendelian inheritance, and the majority of the individuals inherit AMD in a complex multi-gene pattern.

There have been a number of attempts to identify the genes which cause AMD. With the help of the haplotype marker project (HapMap Project), genome wide scanning has identified at least 13 loci linked to AMD on different chromosomes (4-6).

Recently, a polymorphism of complement factor H gene (*Y402H*) was shown to be associated with an increased risk for AMD (7-10). These results were confirmed in many of the countries with large Caucasian populations but not in Japan (11,12). This gene is located on chromosome 1q25-31 where one of the candidate loci was identified by linkage studies. Another recent study reported that a haplotype association of tandemly located complement 2 and factor B was protective for AMD (13).

2.3. Pathology and biochemistry of AMD

The early stage of the dry type AMD is characterized by a thickening of Bruch's membrane, aggregation of pigment granules, and increasing numbers of drusen. The thickening of Bruch's membrane obstructs its function as a 'barrier' between the choroid and the RPE that protects the neural retina from the choriocapillary. Drusen are small yellowish-white deposits that are composed of lipids, proteins, glycoproteins, and glycosaminoglycans. They accumulate in the extracellular space and the inner aspects of Bruch's membrane (Fig. 3). Drusen are not directly associated with visual loss but represent a risk factor for both the dry-type and wet-type AMD. The classification of hard and soft drusen is based on their size, shape, and color; hard drusen are yellowish with diameters <50 μm and are found in eyes that are less likely to progress to advanced stages of the disease, while soft drusen are darker yellow and larger in size, and are found in eyes more likely to progress to more advanced stages of AMD. A small percentage of dry-type AMD patients progress to the late stage of the wet-type AMD that is characterized by geographic atrophy or detachment of RPE and the

development of CNV in the macular region. The presence of a CNV is the factor that most damages the neural retina because the newly developed vessels grow from the choriocapillaris through Bruch's membrane and extend laterally through the RPE cell layer (classic CNV) or extend between the inner Bruch's membrane and RPE (occult CNV). In advanced stages of AMD, the CNV and fluid leaked into the subretinal or intraretinal regions leads to cell death and retinal detachment.

Recent analyses of the progression of drusen have provided important clues that help understand the molecular pathology of AMD. Using both immunohistochemistry and proteomic techniques, the materials in drusen were found to be composed of molecules that mediate inflammatory and immune processes (14, 15). These molecules include components of the complement pathway and modulators of complement activation, viz., vitronectin, clusterin, membrane cofactor protein, and complement receptor-1. In addition, molecules triggering inflammation, viz., amyloid P component, a1-antitrypsin, and apolipoprotein E, were identified in drusen. Cellular debris from macrophages, RPE cells, and choroidal dendritic cells has also been identified in drusen. On the other hand, crystallins, EEFMP1, and amyloid-beta have been found at

higher levels in drusen from individuals unaffected by AMD. The presence of immunoreactive proteins and the oxidative modifications of many proteins in drusen imply that both oxidation and immune functions are involved in the pathogenesis of AMD.

All of these findings suggest that complement activation triggers innate immune responses in the subretinal space. The co-distribution of IgG and terminal complement complexes in drusen indicate that immune responses that directly target antigens in retinal cells might also be occurring. Anti-retinal autoantibodies have been reported in a number of ocular disorders, e.g., macular degeneration in an aged monkey model.

2.4. Animal models for AMD

Access to appropriate biological materials from affected donors at different stages of a disease is an absolute necessity for the study of mechanisms underlying the disease process. However, because it is nearly impossible to obtain retinal tissues from patients or controls, the development of animal models becomes crucial for investigating the biological pathways involved in the progression of the disease and for the

development of therapeutic strategies.

Over the past few years, genetic engineering techniques have generated a number of animal models of AMD in mice, rats, rabbits, pigs, and dogs (16). However in mammals, a well-defined fovea is found only in primates (humans and monkeys), and a search for a monkey line affected with macular degeneration has been persistent for a long time.

A monkey with macular degeneration was first described by Stafford et al in 1974. They reported that 6.6 % of the elderly monkeys they examined showed pigmentary disorders and drusen-like spots (17). El-Mofty et al reported that the incidence of maculopathy was 50% in a colony of rhesus monkeys at the Caribbean Primate Research Center of the University of Puerto Rico (18). At the Tsukuba Primate Research Center (Tsukuba City, Japan), Suzuki et al found a single cynomolgus monkey (*Macaca fascicularis*) in 1986 with a large number of small drusen around the macular region (Fig. 4) (19-21). This single affected monkey has multiplied to a large pedigree of more than 65 affect and 210 unaffected monkeys. Drusen were observed in the macular region as early as one year after birth, and the numbers increased and

spread toward the peripheral retina throughout life. No histological abnormalities have been found in the retina, retinal vessels, or choroidal vasculatures of the eyes with drusen. Immunohistochemical and proteomic analyses of the drusen from these monkeys showed that the drusen were very similar to those in other monkeys with aged macular degeneration sporadically found in older monkeys and also with human drusen (22, 23). These observations by Umeda et al have shown that the Tsukuba monkeys produce drusen that are biochemically similar to those in human AMD patients, but the development of the drusen occurs at an accelerated rate of over 25 times. Currently, 240 loci of the cynomolgus monkey are being investigated to try to identify the disease causing gene and to understand the biological pathways leading to complement activation.

The eyes of monkey are structurally similar to human eyes which make them extremely valuable for macular degeneration studies. However, there are limitations in using this species over other laboratory animals. Monkeys have a relatively longer life span, have a longer gestation period, have a lower birth numbers resulting in a slower rate of expanding the pedigree, more difficult to genetically manipulate, and the cost of

maintenance is high. In the other laboratory animals, the differences in the eye structure, lack of a fovea, and a low cone/rod ratio compared to humans have been considered to be a disadvantage for using them as AMD models. However, they are easier to manipulate genetically and easier and less expensive to maintain. This has made the development of a mouse model of AMD very attractive, and a number of mouse AMD models have been reported recently.

The mouse model described by Ambati et al is deficient either in monocyte chemoattractant protein-1 or its cognate C-C chemokine receptor-2. These mice were found to develop the cardinal features of AMD including accumulation of lipofuscin in drusen beneath the RPE, photoreceptor atrophy, and CNV (24). An impairment of macrophage recruitment allowed the accumulation of C5a and IgG, which leads to the production of vascular endothelial growth factor by the RPE cells and the development of CNVs.

Another mouse model that has three known AMD risk factors: age, high fat cholesterol rich diet, and expression of human apolipoprotein E (apoE2, apoE3, apoE4) has been developed (25). ApoE4-deficient mice are severely affected showing diffuse

subretinal pigment epithelial deposits, drusen, thickened Bruch's membrane, and atrophy, hypopigmentation, and hyperpigmentation of the RPE.

Oxidative stress has long been linked to the pathogenesis of AMD. Imamura et al reported a Cu, Zn-superoxide dismutase (SOD1)-deficient mice that had features typical of AMD in human. Senescent Sod1 (-/-) mice had drusen, thickened Bruch's membrane, and choroidal neovascularization (26). The number of drusen increased with age and also after exposure of young Sod1 (-/-) mice to excess light. The retinal pigment epithelial cells of Sod1 (-/-) mice showed oxidative damage, and their beta-catenin-mediated cellular integrity was disrupted. These findings suggested that oxidative stress may affect the junctional proteins necessary for the barrier integrity of the RPE. These observations strongly suggested that oxidative stress may play a major role in AMD.

The complement components, C3a and C5a, are present in drusen, and were observed in Bruch's membrane of a laser-induced CNV mice model. Neutralization of C3a or C5a by antibody or by blockade of their receptors by a complement inhibitor significantly reduced the CNV. These observations revealed a role for immunological

mechanisms for the angiogenesis and provided evidence for future therapeutic strategies for AMD.

Although the pathology of AMD is pronounced in the macula area, it is not confined to this region. Characteristics of human AMD such as thickening of Bruch's membrane, accumulation of drusen, and CNV have been observed in mouse models. Nevertheless, the primate model will still be the choice for AMD studies, especially at the stage when new therapeutic methods are tested and evaluated for the first time. However, it would be wise and more productive to study both primate and mouse models in AMD research. This will be necessary to learn the mechanisms underlying the disease and to identify clinical and molecular markers for the early stages of AMD. The findings from these studies will provide critical information needed to develop therapies for AMD.

3. Glaucoma

3.1. Overview of Glaucoma

Glaucoma is a heterogeneous group of complex neurodegenerative disorders

that is characterized by the constriction of the visual field, death of retinal ganglion cells (RGCs), and a pathognomonic deformation of the optic nerve head (ONH) known as glaucomatous cupping. Glaucomas are classified into three main types: open-angle, closed-angle, and congenital glaucoma. Each of these types is subdivided into primary and secondary types.

3.2. Epidemiology and Genetics of Glaucoma

Primary open-angle glaucoma (POAG) is the most common form of glaucoma, and occurs in about 4.5 million people worldwide and accounts for 12% of all global blindness. By the year 2020, over 11 million people have been predicted to be blind from primary glaucoma (28). POAG is often, but not always, associated with elevated intraocular pressure (IOP), which is one of the main risk factors in glaucoma. However, about a third of all patients with POAG develop the disease without an IOP elevation, and in these patients, the IOP is continuously below 21 mmHg. This form of POAG is called normal tension or low tension glaucoma (NTG). A reduction of the IOP, even in cases of NTG, is the main, clinically-proven, treatment for glaucoma.