·Commentary·

Development of age-related macular degeneration experimental models

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Received: 2009-03-22 Accepted:2009-08-08

Abstract

• We reviews different experimental models of age-related macular degeneration (AMD) used in recent studies. The most widely used one is Laser-induced choroidal neovascularization (CNV), which represents the late severe stage in the exudative form of AMD. Other models are based on several different pathogenesis, like geographic atrophy, drusen formation or multifactorial effects, like age, light, high fat, *etc*. It is hoped that this article could become a good reference for researchers who need to choose suitable models for AMD study.

• KEYWORDS: age-related macular degeneration; model

Jiang W, Chiou GCY. Development of age-related macular degeneration experimental models. *Int J Ophthalmol* 2009;2(3):189-194

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of poor vision among people aged over 65. It has many clinicopathologic signs, numerous pathogenesis and various etiologies. The treatment for this disease is limited. Many researches studying this disease have established some experimental models both *in vivo* and *in vitro*. The basic foundation of these models was based on etiology or acology, which could also be classified into two types:wet- and dry-AMD. There is no perfect model representing exactly what really happened in the patient's eyes. Moreover, what happened in one kind of model usually can not be used to explain a single theory either. In this review, we try to introduce these experimental AMD models on the bases of pathology, pathogenesis, etiology, and others.

PATHOLOGY OF AMD

Neovascularization Choroidal neovascularization (CNV) is the leading cause of wet-AMD, which affects mostly the visual acuity. There are different methods available to induce CNV in experiments. The most commonly used one is the laser beam. The purpose of this model is to perforate the Bruch's membrane to trigger CNV formation. Using a slit lamp delivery system, 2-8 spots are placed through lens at equal distance around the optic discs with acute vapour bubbles. Those spots having no bubble or having subretinal hemorrhage are excluded. Animals used can be rats, mice or pigs. The laser used can be krypton red laser [1], green argon laser [2,3] and diode laser [4-6]. These methods can develop stable CNV quickly.

Vascular endothelial growth factor (VEGF) is closely related to the formation of CNV[7]. Thus, some methods were based on the increasing levels of VEGF in the eyes. One is using adeno-associated viral vector encoding human VEGF165 injected into the subretinal space(SRS) of Sprague-Dawley or Long Evans rats, in which the CNV persisted for more than 20 months [8]. Schwesinger *et al* [9] established a transgenic murine model through the overexpression of VEGF by the retinal pigment epithelium to induce CNV. They introduced a tissue-specific murine retinal pigment epithelium promoter coupled with murine VEGF164 cDNA with a rabbit b-globin-3 UTR into the genome of albino mice. The expression of VEGF protein was increased in both retinal pigment epithelium and choroids, with the increase of intravascular adherent leukocytes and vessel leakage.

Another transgenic mouse model is prokineticin 1 (hPK1), a mitogen of fenestrated endothelium. They generated transgenic mice with an expression of hPK1 in the retina

using rhodopsin promoter. Consequently, enlarged vascular bed of choroid, resembling CNV, was observed without any morphological changes in the retinal vasculature. The major fluorophore of lipofuscin was highly accumulated in the transgenic mouse eyes as compared to controls[10].

Neovascularization can be easily formed by ischemia. A hyperoxia-induced retinal ischemia and neovascularization occurred in all mice's eyes by means of putting one-week-old mice into 750mL/L O_2 for 5 days, from postnatal day 17 (P17) to postnatal day 21 (P21), and then into room air [11]. The time exposed to oxygen could be changed from P7 to P12 or from within 2 hours of birth to P14 [12]. The concentration of the oxygen could be changed into 730mL/L [13] or 24 hours alternating cycles between 500mL/L O_2 and 100mL/L O_2 [12]. This type of neovascularization develops in the retina, but not in choroids.

An isolated neovascular structure model could be obtained by corneas consisting of an easily accessible monolayer-like neovascular net within a transparent matrix [14]. Using this model, researches could evaluate the efficacy of drugs or photodynamic therapy on ocular neovascularization [15,16]. Another kind of isolated neovascular structure is the highly vascularized 8 to 9-day-old chicken chorioallantoic membrane, which is similar to the rapid growth of blood vessels in the wet form of AMD[17].

Based on above-mentioned models, some important AMD related data could be generated, with mouse serum albumin (MSA), carboxyethylpyrrole (CEP)-MSA, or VEGF[18].

In vitro experiment, using choroidal endothelial cells (CECs) which are involved in the process of CNV formation is a good model for evaluating the anti-proliferation effect by drugs[19].

Drusen Drusen is a typical clinicopathologic entity in nonexudative macular degeneration, which is caused by the change of retinal pigment epithelium and Bruch's membrane. There is a spontaneous model of Rhesus macaques (Macaca mulatta) for age related macular drusen. The prevalence and severity of drusen formation in their eyes are linearly related to increasing age and are significantly higher in specific maternal lineages (matrilines) [20]. Several researches used this model for studying the mechanism and treatment of AMD[21, 22].

Basal Laminar Deposit Similar to drusen, basal laminar deposit(BLD) is another typical signal of dry-AMD development and led by extracellular deposits. BLD is located between the cell membrane of the retinal pigment epithelium (RPE) and its basement membrane, while drusen is located in between the basement membrane of the RPE and the remainder of Bruch's membrane, or external to Bruch's membrane[23,24]. An experiment showed that transgenic mice expressing the human apolipoprotein-E (apo-E) 3-Leiden gene (and producing a dysfunctional form of human apo-E3) on a high fat/cholesterol (HFC) diet or on a normal mouse fed for 9 months. All eyes of the apo-E3-Leiden mice on an HFC diet contained BLD (class 1 to class 3), whereas two of six apo-E3-Leiden mice on normal chow showed only BLD class 1. So these apo-E3- Leiden mice can be used as animal model for studying the pathogenesis of BLD which could be enhanced by HFC diet[25].

RPE Cells Retinal pigment epithelial (RPE) cells are very important in eye's physiological function. Some considered that AMD is caused by loss or disfunction of RPE cells which leads to abnormal build-up of photoreceptor outer segment breakdown products. A transgenic mouse line (mcd/mcd) expressed a mutated form of cathepsin D which is enzymatically inactive thus impairing processing of phagocytosed photoreceptor outer segments in the RPE cells. Histological studies showed proliferation of RPE cell, degeneration of photoreceptor, shortening of photoreceptor outer segments, and accumulation of immunoreactive photoreceptor breakdown products in the RPE cells[26].

Pigmented dystrophic Royal College of Surgeons (RCS) rats have been widely used by various researches, because most photoreceptors of these rats die during the first three months of life. Due to a genetic defect in photoreceptor outer segment, and phagocytosis by the adjacent RPE, the RCS rats could be used for the treatment research on RPE protection and on AMD improvement[27]. Another experiment for RPE degeneration uses a single injection of sterile 1% solution of $NaIO_3$ in saline into the tail vein. The final dose used was 50mg/kg body weight, which could produce a progressive degeneration of the RPE and the neural retina[28].

If the retinal pigment epithelium is debrided in the porcine eye, atrophy of the choriocapillaris appeared within 1 week. The method uses pars plana vitrectomy firstly, then, creating neurosensory retinal detachments by injecting mitomycin C and edetic acid into the subretinal space. Twenty minutes later, the retinal pigment epithelium is debrided, and the retina

is reattached with a fluid-gas exchange. So this model could be used for nonexudative age-related macular degeneration[29]. In *in vitro* experiments, RPE cells were widely used [30], which could be seen as the outer blood-retinal barrier when they grow on coated flasks[31] and in which sub-RPE deposits could be assessed by machines such as electron microscopy[32].

PATHOGENESIS OF AMD

Oxidative Damage Oxidative stress, having long been linked to the age-related and degenerative diseases, is implicated in the pathogenesis of AMD. Mice deficient in Cu, Zn-superoxide dismutase (SOD) of different ages showed that the older animals had drusen, thickened Bruch's membrane, and choroidal neovascularization. The number of drusen increased with age, and if exposing of young SOD1 knock out mice to excess light, drusen could be induced and RPE cells could be oxidatively damaged [33]. Because the oxidative damage is likely to be the photoreactive pigments which accumulate progressively and constitute the lipofuscin of RPE cells. RPE cells are always used in *in vitro* experiments[34].

Using hydroquinone (HQ) in cultured RPE could upregulate nonlethal blebbing and decrease extracellular matrix (ECM) turnover. Animals exposed to oral HQ induced nonlethal bleb injury and sub-RPE deposits. These results were considered as nonlethal oxidant injury induced by HQ[35].

Complement Component Some works demonstrated that complement components C3 and C5 are constituents of drusen in patients with AMD. Mice deficient in monocyte chemoattractant protein-1 (Ccl-2; also known as MCP-1) or its cognate C-C chemokine receptor-2 (Ccr-2) developed cardinal features of AMD, including accumulation of lipofuscin in drusen and in beneath the RPE plus photoreceptor atrophy and CNV[36].

Lipid Accumulation Lipids could be involved in many aging diseases. An experiment using LDL receptor deficient mice (an atherosclerotic murine model) being fed with standard or a high fat (HF) diets was used to study the changes on mice's eyes. They found lipid particles accumulated in Bruch's membrane (BrM) which was further increased after fat intake. VEGF expression was found in the outer retinal layers and appeared to correlate with the amount of lipid particles present in BrM[37].

Iron Ferroxidase ceruloplasmin (Cp) and/or hephaestin (Heph) deficient mice had been used to study the effect of iron on the development of AMD. These mice had age-dependent RPE hypertrophy, hyperplasia and death coupled with photoreceptor degeneration and subretinal neovascularization, providing a model for some features of the human retinal diseases aceruloplasminemia and age-related macular degeneration[38].

ETIOLOGY OF AMD

Age and High-fat Diet Advanced age is considered as the first etiology in AMD, which have been confirmed by many clinic statistics and researches. Besides, epidemiologic data also indicated that dietary fats, especially polyunsaturated fats, were associated with AMD [39]. There were also some related researches which showed that only old age (male 65-123 weeks, female 75-127 weeks) or high-fat chow (Diet 5015; PMI Nutrition International Test Diet) could elicit pathogenesis of AMD in mouse's eyes[40, 41].

Light Light exposure, inducing oxidative damage, is suspected to play an important role in the etiology of AMD. Different kind of lights were used on animal eyes, such as blue light (14μW/cm^2; bandwidth, 390 to 430nm)[42], nonphototoxic levels of argon laser 488nm blue-green light [41] and 15 000 lux of diffuse white fluorescent light (TLD-36 W/965 tubes, Philips; ultraviolet-impermeablediffuser). The last one was used for 2 hours on dilated pupils of already dark-adapted animals by being maintained in dim red light, those animals were kept in darkness all the time until analysis[43].

Smoke and Alcohol Cigarette smoke has been indicated by epidemiologic studies that it is the single greatest environmental risk factor for both dry- and wet-AMD [44]. One experiment [45] was conducted by exposing mice to inhaled cigarette smoke by using of a custom-built microprocessor-controlled cigarette-smoking machine or by feeding mice with a defined cigarette smoke component, HQ, as mentioned above [35]. The results of this experiment showed the formation of sub-RPE deposits, thickening of Bruch's membrane, and accumulation of deposits within Bruch's membrane. On another experiment, mice were fed with nicotine in their drinking water (100μg/mL) for 4 weeks, the nicotine increased size and severity of experimental CNV in this mouse model[46]. In order to investigate whether alcohol influences the development of CNV, Bora *et al*[47] gave 8g/kg alcohol and regular diet to rats for 10 weeks. The result showed that fatty acid ethyl ester synthase (FAEES) activity was increased 4.0-fold in the choroid of

alcohol-treated rats as compared with controls. The amount of ethyl esters produced in the choroid of 10 weeks alcohol-fed rats was 7.4-fold more than rats fed alcohol for 1 week. And the size of CNV induced by laser treatment increased by 28% in alcohol-fed rats.

Gene Beside environmental factors, gene also plays an important role in the development of AMD. Thus, some models used are transgenic animals based on different etiology. Eyes of apolipoprotein B100 (APO B100) transgenic mice treated with blue-green light showed a high frequency of "moderate BLD", whereas the nonexposed eyes did not [41]. Among eyes of aged, targeted replacement mice expressing human apoE2, apoE3 and apoE4, apoE4 mice were the most severely affected, which developed a constellation of changes that mimic the pathology associated with human AMD [40]. Fas (CD95)-deficient (lpr) and FasL-defective (gld) mice had a significantly increased incidence of neovascularization. In gld mice there was massive subretinal neovascularization with uncontrolled growth of vessels.

Cultured choroidal endothelial cells were induced to undergo apoptosis by retinal pigment epithelial cells through a Fas-FasL interaction[48].

OTHERS

Physical Method Matrigel, a basement membrane extract which is solidifiable after implantation in tissue could induce neovascularization and focal retinal degeneration after subretinal injected in mice[49].

Other Diseases Since the clinical manifestation and pathology of some other diseases are similar to AMD's, researchers could use these models to investigate AMD. For example, Stargardt macular dystrophy (STGD), which is characterized as AMD by the accumulation of high levels of lipofuscin in the retinal pigment epithelium. It precedes to degeneration of the photoreceptors in the macula and atrophy of RPE, just like the transgenic ELOVL4 Mice[50,51]. Different from STGD which likes dry-AMD, Sorsby's fundus dystrophy (SFD) is a rare autosomal dominant disorder that results in degeneration of the macular region of the retina and leads to the rapid loss of central vision likes wet or exudative form of AMD[52].

CONCLUSION

Age-related macular degeneration (AMD), the most common cause of blindness in the elderly in developed countries, has a complex aetiology with both genetic and environmental factors playing roles. It also has a complex pathology and a series of clinical features. Till now, there has no proper model which could reflect all of the related factors. The established models could only partly represent the development of AMD. Laser-induced CNV is the most commonly used. This method perforates the Bruch's membrane so that CNV could be easily and quickly developed. However, not only Bruch's membrane, retina and part of choroid could also be damaged around the laser spot, which obviously doesn't mimic completely the pathology of clinical AMD. Furthermore, although there are many experiments using mice and rat, these animals have no macula at all. So when we want to investigate action mechanisms or treatments of AMD, we should choose the most closely related model not just one kind of model. Besides, how to evaluate the development of pathological changes and to study the effect of different treatments are other important topics. The best we can do today is to pick the best model which mimic human AMD and show the quantitative changes, even though, we might loose some important data or might be still far away from clinical reality. Therefore, new animal models still need to be created.

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