Chapter 33
Retinal Neovascular Disorders: Mouse Models for Drug Development Studies

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Keywords Retinal neovascularization • Neovascular disorders • Age-related macular degeneration • Diabetic retinopathy • Retinopathy of prematurity • Mouse models • Drug development

33.1 Introduction

Neovascularization is a hallmark of several eye diseases leading to visual impairment, and its epidemiological impact is substantial (Lee et al. 1998). In retinal degenerative disease models, neovascularization is the process by which the choroid and/or retina become infiltrated with new blood vessels. In retinal neovascularization (RNV), sprouting retinal vessels penetrate the inner limiting membrane (ILM) and grow into the vitreous, and in some cases, grow through the avascular outer retina into the subretinal space (Campochiaro 2000). Numerous clinical and

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M.M. LaVail et al. (eds.), *Retinal Degenerative Diseases*, Advances in Experimental Medicine and Biology 723, DOI 10.1007/978-1-4614-0631-0_33,

experimental observations indicate that ischemia (or hypoxia) is the driving force behind RNV (Michaelson and Steedman 1949). Occlusion of retinal vessels leading to ischemia is a feature of diseases with RNV, including diabetic retinopathy (DR) and retinopathy of prematurity (ROP) (Campochiaro 2000).

Choroidal neovascularization (CNV) is characterized by new blood vessels emanating from the choroid into the subretinal pigment epithelium, subretinal space, or both (Qazi et al. 2009).This leads to the formation of neovascular membranes, which include vascular endothelial cells, retinal pigment epithelial (RPE) cells, and macrophages (Das and McGuire 2003). Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are essential mediators in the development of CNV, which is the primary form of neovascularization in age-related macular degeneration (AMD) patients. Although multiple factors have been implicated in the development of neovascularization, future studies are warranted in the identification of novel treatment strategies. To better understand the current mouse models available to study RNV and CNV, a brief review of the retinal diseases characterized by neovascularization is examined below.

33.2 Proliferative Diabetic Retinopathy

The most common cause of blindness among Americans between the ages of 20–74 is DR. This disorder can be classified as either nonproliferative or proliferative. Whereas nonproliferative DR is characterized by venous dilation, retinal hemorrhage, and microaneurysms, proliferative DR is characterized by abnormal new vessel and fibrous tissue proliferation on the surface of the retina (Lee et al. 1998). Ischemia of the inner retinal layers causes the release of VEGF, stimulating new blood vessel formation locally and in other regions of the eye (Besirli and Johnson 2009). For diabetics, RNV is the major contributor to blindness, and treatment of neovascularization can reduce severe visual loss from 50% to approximately 5% per year (Lee et al. 1998).

33.3 Retinopathy of Prematurity

ROP is characterized by RNV that eventually affects the vitreous, causes retinal detachment, and inevitable blindness in premature infants (Lee et al. 1998). ROP, like DR, is primarily characterized by RNV and less by CNV. The disease can progress in two phases: (1) delayed retinal vascular growth after birth and partial regression of existing vessels followed by (2) hypoxia-induced pathological vessel growth (Chen and Smith 2007). ROP develops through conditions of excessive hyperoxia and hypoxia leading to deregulation of VEGF expression. Serum insulin-like growth factor 1 (IGF-1) has also shown to directly correlate with the severity of ROP in the clinic. Taken together, the pathophysiology of ROP is similar to that of other retinal disease with RNV.

33.4 Age-Related Macular Degeneration

A multifactorial cause of photoreceptor degeneration, AMD accounts for more than half of all blindness and visual impairment in developed countries primarily affecting individuals over 60 (Ting et al. 2009). Patients with AMD are categorized as having nonexudative (dry) or exudative (wet) stage forms of the disease. Dry AMD patients exhibit large, poorly demarcated drusen and RPE abnormalities. Wet AMD is characterized by local regions of RPE atrophy and growth of new blood vessels from the choroid that penetrate Bruch's membrane and enter the retina where they can leak and cause damage. Genetic association studies on AMD patients identified Complement Factor H (CFH) and Age-related Maculopathy Susceptibility 2 (ARMS) genes as contributors to AMD (Wright et al. 2010), with the effect sizes of these two susceptibility alleles unusually large by the standard of most complex traits. Current successful therapeutic strategies for treating AMD and improving visual quality include the use of anti-VEGF medicines.

33.5 Genetic Mouse Models for Neovascularization

The distinction between RNV and CNV in mouse models is important in that they are used to determine the type of disease for which they are most appropriate. For example, DR and ROP are both primarily characterized by RNV, and AMD, which may also be associated with RNV, exhibits CNV as the primary mechanism for neovascularization in the eye.

Transgenic mouse models for RNV provide an adequate resource for the characterization of this phenomenon. One example is the *rho*/*VEGF* transgenic mouse, which develops retinal and subretinal neovascularization (SRN) as a consequence of VEGF expression driven by the rhodopsin promoter. This model is a close representation to patients with retinal angiomatous proliferation (RAP) (Miller 1997). The development of $VEGF_{165}$ overexpressing transgenic mice driven by the truncated rhodopsin promoter developed phenotypes ranging from mild to severe RNV and are also used in RNV studies (Miller 1997).

The reverse tetracycline transactivator (rtTA) inducible promoter system coupled with the rhodopsin (*rho*/*rtTa-TRE*/*VEGF*) or interphotoreceptor retinoid-binding protein (IRBP) promoter (*IRBP*/*rtTA-TRE*/*VEGF*) has been used to control the onset of VEGF expression in the retina (Grossniklaus et al. 2010). The addition of doxycycline in these mice activates the expression levels of VEGF, and subsequently causes extensive RNV. In comparison to *rho*/*VEGF* mice, these transgenic models are associated with total retinal detachment and higher ocular levels of VEGF mRNA and protein (Ohno-Matsui et al. 2002).

A recently identified knockout mouse that also exhibits RNV is the $Nrl^{-/-}Grk1^{-/-}$ mouse. The neural retina leucine zipper knockout ($Nrl^{-/-}$), which lacks the transcription factor responsible for normal rod photoreceptor development, leads to a retinal

phenotype with all cone photoreceptors that include an enhanced number of S-cones and a normal number of M-cones. The double knockout that lacks the G-protein-coupled receptor kinase 1 gene (*Nrl*$^{-/-}$*Grk1*$^{-/-}$) involved in phototransduction recovery exhibits a light independent age-related degeneration (Zhu et al. 2003, 2006). These mice exhibit an RNV phenotype similar to RAP and retinal vascularization that is first observed at 1 month and is mediated by the inflammatory response (Yetemian et al. 2010; Yetemian 2010).

Relevant models of CNV can be found in transgenic knockout mice where CNV is a phenotypic distinction. One such model is the monocyte chemoattractant protein-1 (Ccl2) or its receptor CC-chemokine receptor-2 (Ccr2) deficient mouse, which are both current models for AMD (Ambati et al. 2003). These transgenic mice lacking either Ccl2 or Ccr2 fail to recruit macrophages to the RPE or Bruch's membrane, thereby allowing the accumulation of complement factor C5a and IgG, both of which induce VEGF production (Ambati et al. 2003).

Other transgenic knockout mice that exhibit a CNV phenotype include mice that overexpress Apolipoprotein E (ApoE) that were fed a high fat diet and developed AMD like lesions (Malek et al. 2005). Disruption of ceruloplasmin and hephaestin in mice causes iron overload and subsequent AMD-like changes. These mice develop RPE abnormalities and photoreceptor degeneration (Hahn et al. 2004). Knockout mice for the very low density lipoprotein receptor gene (Vldlr^{TM1Her}) develop new blood vessels in the outer plexiform layer (OPL) of the retina as well as choroidal anastomoses by 3 months (Heckenlively et al. 2003). The transgenic mouse line (mcd/mcd) exhibits features associated with geographic atrophy and AMD. The mcd/mcd mice express a mutated form of cathepsin D that is enzymatically inactive, thereby impairing photoreceptor outer segment phagocytosis by RPE cells (Rakoczy et al. 2002). Also, the Cu, Zn superoxide dismutase-deficient mouse (*Sod*$^{-/-}$) has been shown to exhibit fundus and histological evidence of CNV in approximately 10% of *Sod*$^{-/-}$ mice.

Another model for neovascularization similar to AMD is the spontaneous autosomal semidominant mouse mutation Belly spot and tail (Bst), which arose in the C57/Bl6J inbred strain at the Jackson Laboratory (Smith et al. 1999). The mutation was mapped to chromosome 16, 1.9 ± 1.1 cM from *D16Mit168*, and these mice have late closure of the optic fissure, delayed retinal differentiation, decreased number of retinal ganglion cells, and coloboma of the optic nerve and retina (Smith et al. 1999). A highly variable phenotype, aging *Bst*+ mice were found to have SRN. SRN begins in the choroid with vascular invasion through Bruch's membrane into the subretinal space, and can lead to CNV in the form of subretinal hemorrhage that may undergo fibrosis and cause more retinal damage (Smith et al. 1999)

33.6 Nongenetic Models for Neovascularization

Animal models for the study of RNV include hypoxia-induced, vascular occlusion, and intraocular injection of pro-angiogenic molecules. Hypoxia-induced models have been developed in several species and is performed by exposing mice to hyperoxia, then placing them in normoxic conditions, causing an ischemic situation that

initiates rapid abnormal neovascularization. This model has proven to delineate the molecular changes in neovascular eye disease and directly correlates to ROP (Ashton 1966; Chen and Smith 2007). Occlusion of retinal veins by laser photocoagulation or photodynamic therapy has also been used in RNV studies (Ham et al. 1997).

Animal models for nongenetically induced CNV include laser and surgically induced models. Experimental laser-induced mice were created using spot treatments from a krypton laser to create photocoagulation injuries to Bruch's membrane (Ryan 1979; Tobe et al. 1998). The laser-induced model is currently well established and is also used in preclinical trials for the study of anti-angiogenic drugs. Although the physical insult to Bruch's membrane differs from the long-term chronic conditions that occur in human AMD, the models closely mimic natural cellular responses that occur in human CNV. Surgically induced forms of CNV are also currently in practice and are done primarily by the injection of synthetic peptides, viral vectors containing VEGF, and inert synthetic materials (Grossniklaus et al. 2010).

33.7 Use of Mouse Models for Neovascularization in Preclinical Drug Testing

The discovery that VEGF plays a significant role in neovascularization spurred the development of several anti-VEGF pharmacological treatments such as Bevacizumab (Avastin), Ranizumab (Lucentis), (Genentech, Inc.), and Pegaptanib (Macugen) (Eyetech, Inc.). Bevacizumab (BVZ) is a full-length humanized monoclonal antibody that binds all isoforms of VEGF-A, Ranizumab (RBZ) is the 48 kDa form of BVZ, and Pegaptanib is a 28-base ribonucleic acid aptamer that binds to $VEGF_{165}$. All are FDA approved, and the extensive preclinical studies for each involved the use of some of the mouse models discussed above. A study by Katsuaki et al. compared the effects of intraocular RBZ and BVZ injections using *rho*/*VEGF* and doxycycline-treated *Tet*/*opsin*/*VEGF* mice to demonstrate not only safety but also efficacy in suppression of SRN (Katsuaki et al. 2009). A study funded by Eyetech, Inc. used a murine model of ROP in their preclinical testing for Pegaptanib, among several other animal models for RNV (Eyetech Study Group 2002). It is apparent from the preclinical testing conducted on these three currently marketed treatments for AMD that the mouse models utilized were influential in the development of these drugs and will continue to be utilized during the development of future treatments for neovascularization.

33.8 Conclusion

Despite their similarities in structure and function, the retinas of humans and mice are quite unique and do present challenges when compared. Mice, for example, have only two opsin expressing cones when compared to humans and lack a defined

macula. Nevertheless, they have similar morphology and neuronal structures that are extremely comparable to the human retina and are extensively used in many disease studies. DR, ROP, and AMD are the leading forms of retinal dystrophy in the aging human population, and mouse models are becoming increasingly useful in identifying causative mechanisms and therapeutic targets to protect against these diseases.

As we learn more about neovascularization, we will continue to develop and explore new animal models for the treatment and prevention of disease. This short review is not a comprehensive study on all the available mouse models for preclinical testing, but it summarizes significant trends and variability in RNV research and the high impact mouse models have in human disease studies. Mouse models are crucial to understanding the molecular and cellular mechanism behind retinal degeneration and will continue to provide avenues for potential therapeutic advancement.

Acknowledgments We thank members of the Mary D. Allen Laboratory for scientific discussions. CMC is the Mary D. Allen Chair in Vision Research, DEI, and a Research to Prevent Blindness (RPB) Senior Scientific Investigator. This work was supported, in part, by NIH Grant EY015851 (CMC), EY03040 (DEI), RPB (DEI & CMC), Dorie Miller, William Hansen Sandberg Memorial Scholarship (RMY), Tony Gray Foundation, Mary D. Allen Foundation (Dr. Richard Newton Lolley Memorial Scholarship [RMY]), and an RD2010 Travel Award (RMY).

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