

Ocular Neovascularization: In Vitro and In Vivo Models

The development of these models provides researchers with an important tool for studying the angiogenic process.

BY JOHN S. PENN, PHD, AND SUSAN E. YANNI

Many pathological processes are characterized by persistent abnormal angiogenesis. Pathological angiogenesis in the eye (ie, ocular neovascularization) is the leading cause of blindness in the developed world. A thorough understanding of the mechanisms that stimulate ocular neovascularization, therefore, is necessary to develop viable chemotherapies. The development of in vitro and in vivo models of ocular neovascularization has provided researchers with an important tool for studying the angiogenic process.

BENEFITS OF MODELS

In vitro models of ocular neovascularization are beneficial in that they limit the use of animals, are high throughput, and allow for a tightly controlled experimental environment. These strengths allow researchers to distill, define, and understand discrete components of the angiogenic cascade.

Of the growth factors involved in retinal angiogenesis, vascular endothelial cell growth factor (VEGF) is thought to be a principal mediator.¹ In response to retinal hypoxia, several cell types exhibit an increased production of VEGF (eg, Müller cells, retinal pigment epithelial [RPE] cells, astrocytes). These cells provide an appropriate means to study mechanisms of hypoxia-induced VEGF production. VEGF receptors are located on the membranes of microvascular endothelial cells.² Retinal microvascular endothelial cells are the primary component of retinal neovascularization, and provide an appropriate method of studying VEGF signal transduction intermediates and angiogenic cell behaviors.

It is important to note that the value of these cell-

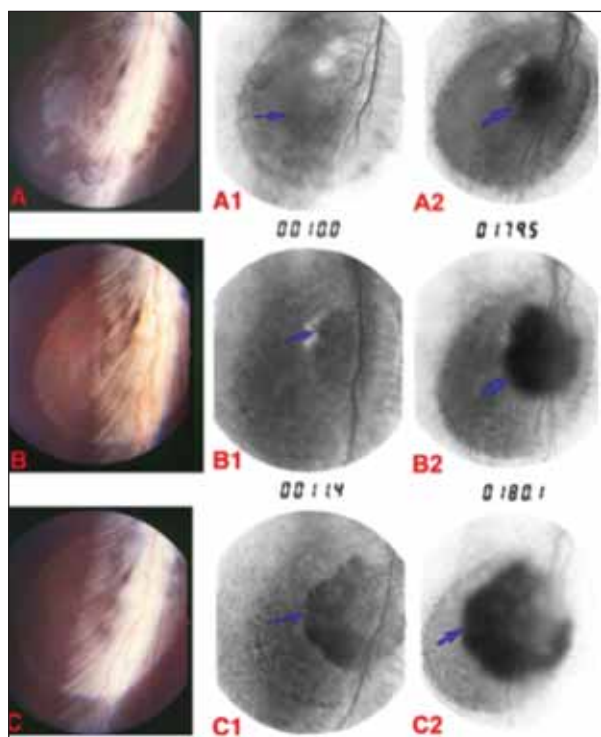


Figure 1. Laser-induced choroidal neovascularization (CNV) in rabbit eyes.

based models, which are used to define mechanisms of the angiogenic cascade and in the screening phase of drug development, is dependent upon choosing the correct cell type and bioactivity model. Even then, in vitro models lack many of the paracrine influences that exist in the cell's native environment and in the disease condition.

The development and use of various in vivo models

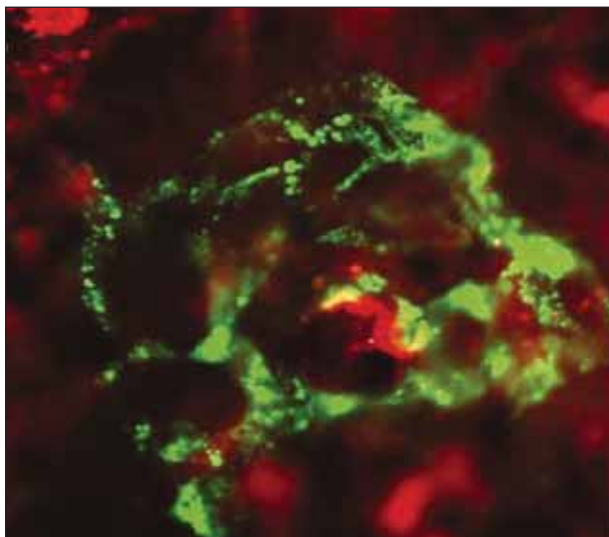


Figure 2. Laser-induced CNV in a mouse model.

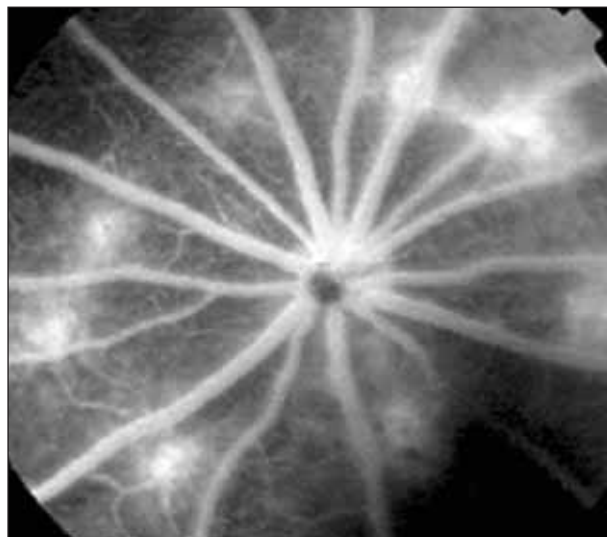


Figure 3. Laser-induced CNV in a rat model.

of ocular neovascularization have become the standard method of testing the efficacy of preclinical antiangiogenic compounds. The most widely used of these models include the corneal micropocket assay, rodent models of oxygen-induced retinopathy (OIR), and models of laser-induced choroidal neovascularization (CNV) (Figure 1). Each of these models carries advantages and disadvantages.

CORNEAL MICROPOCKET ASSAY

The corneal micropocket involves surgically implanting a VEGF—or other angiogenic growth factor-laced pellet that stimulates neovascular growth originating from the limbal area of the cornea, into the corneal stroma. Because the cornea is readily accessible for observation, the assay permits a noninvasive assessment of the neovascular response. This makes the corneal micropocket assay ideal for long-term studies. The greatest disadvantage of the model is the fact that the cornea is an avascular tissue. This means that for neovascularization to occur, there must be exogenous stimulation. The use of an avascular tissue and exogenous stimulation in an angiogenic assay calls into question the relevancy and translational capacity of the assay. Additionally, the assay requires a technically demanding surgical procedure.

MOUSE MODEL

The mouse model of OIR, initially developed to study the pathogenesis of retinopathy of prematurity (ROP), is a well-characterized model, demonstrating

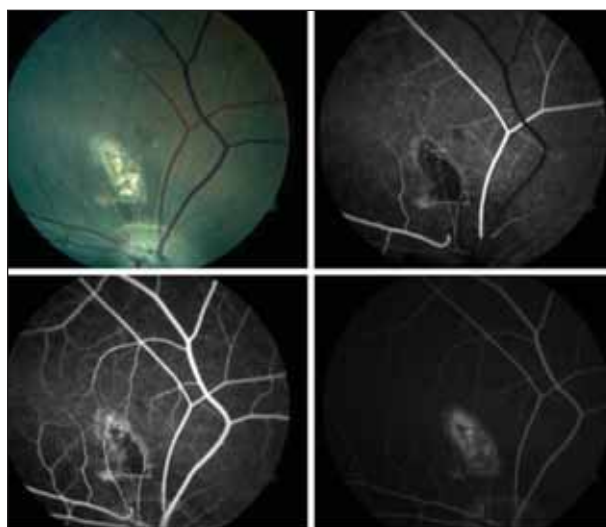


Figure 4. Laser-induced CNV in pig eyes.

rapid, measurable, and reliable pathology. Other strengths of the model are that mice (1) produce relatively large litters, (2) are relatively inexpensive to house, and (3) neovascularization develops fairly rapidly. The process is slow enough, however, that early and late events in the angiogenic cascade can be discriminated. The ability to manipulate the genome of the mouse is one of this model's greatest strengths, which permits the researcher to more precisely define and characterize the role of various genes involved in the angiogenic response.

This model suffers two significant disadvantages. In order to induce pathology, mice must be exposed to clinically irrelevant oxygen levels far above those used to treat premature infants. In addition, mice exposed to this model do not produce a human-like pattern of pathology.

RAT MODEL

The rat model ROP has many of the advantages of the mouse model of OIR: it is a well-characterized model, demonstrating rapid, measurable, and reliable pathology; rats produce large litters; are relatively inexpensive to house; and the model requires no exogenous stimulation of neovascularization. An attractive feature of this model is that rats are exposed to clinically relevant oxygen levels, which produces a human-like pattern of pathology. Manipulation of the rat genome has been, and continues to be, however, difficult to achieve.

LASER MODEL

The laser-induced model of CNV is a highly reproducible model that mimics many features of CNV occurring in the wet form of age-related macular degeneration (AMD), the leading cause of blindness in the elderly. This model is the mainstay of preclinical drug testing for subretinal CNV (Figures 2-5). The model exhibits a number of features of AMD-related CNV including (1) the penetration of Bruch's membrane by choroidal capillaries, (2) accumulation of subretinal fluid, (3) leukocyte congregation, (4) fibrovascular scarring, (5) recruitment of macrophages, and (6) increased VEGF within the RPE. On the other hand, laser-induced lesions more accurately model a wound-healing response rather than true CNV lesions due to AMD. As the laser-induced wound heals, the proangiogenic factors decrease. This is, the opposite of what occurs in wet AMD, where the CNV is sustained and typically evolving. Additionally, and in contrast to the human condition, the model produces nonspecific local inflammatory reactions secondary to laser treatment.

Although this model has contributed significantly to our understanding of CNV in AMD, it is often associated with technical artifacts (eg, glially derived subretinal fibrovascular membranes), and in the end,

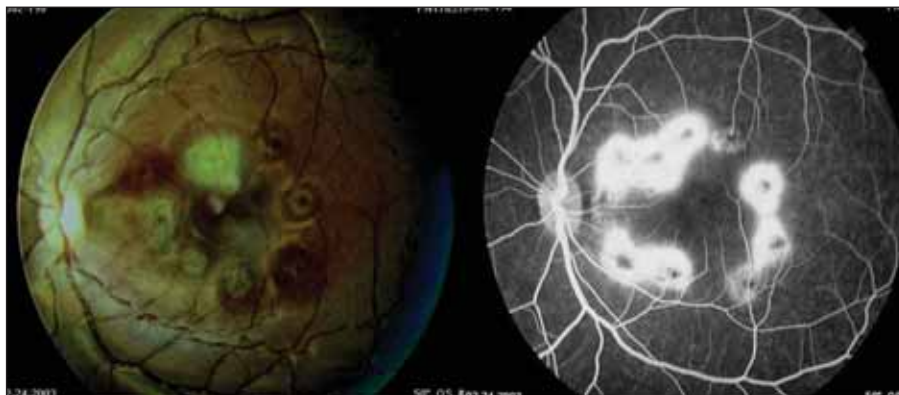


Figure 5. Laser-induced CNV in primate eyes.

acute laser injury does not mimic the chronic disease condition.

STRENGTHS AND WEAKNESSES

Each of these models has been developed for multiple species, and each species has its own strengths and weaknesses. The evaluation of the species-specific behaviors within a model, and how well they mimic the disease process under study dictates the choice of species that is used, and contributes to the relevancy of the model for a proposed study.

The development of in vitro and in vivo models of ocular neovascularization development has provided researchers with important tools for studying the angiogenic process. These models have been developed in more than one species and involve a variety of mechanisms of induction, pathology, and assessment. Unfortunately, no single model can adequately predict therapeutic potential in humans. This means that more than one must be employed in preclinical testing. Clearly, the need remains for researchers to continue to refine the currently existing models and to develop appropriate new models of ocular angiogenesis. ■

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