Animal models of diabetic retinopathy

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Abstract: The retina is the posterior neuro-integrated layer of the eye that conducts impulses induced by light to the optic nerve for human vision. Diseases of the retina often leads to diminished vision and in some cases blindness. Diabetes mellitus (DM) is a worldwide public health issue and globally, there is an estimated 463 million people that are affected by DM and its consequences. Diabetic retinopathy (DR) is a blinding complication of chronic uncontrolled DM and is the most common cause of blindness in the United States between the ages 24–75. It is estimated that the global prevalence of DR will increase to 191.0 million by 2030, of those 56.3 million possessing vision-threatening diabetic retinopathy (VTDR). For the most part, current treatment modalities control the complications of DR without addressing the underlying pathophysiology of the disease. Therefore, there is an unmet need for new therapeutics that not only repair the damaged retinal tissue, but also reverse the course of DR. The key element in developing these treatments is expanding our basic knowledge by studying DR pathogenesis in animal models of proliferative and non-proliferative DR (PDR and NPDR). There are numerous models available for the research of both PDR and NPDR with substantial overlap. Animal models available include those with genetic backgrounds prone to hyperglycemic states, immunologic etiologies, or environmentally induced disease. In this review we aimed to comprehensively summarize the available animal models for DR while also providing insight to each model's ocular therapeutic potential for drug discovery.

Keywords: Diabetes mellitus (DM); diabetic retinopathy (DR); animal model

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia that increases the stress within microvasculature of many organ systems including the eye (1). Diabetic retinopathy (DR) is a major complication of DM and is the most common cause of blindness in the United States between the ages 24–75 (2,3). The development of DR is directly correlated to the duration of hyperglycemia (4,5), and the disease stages of DR follow a progression of blood vessel damage. Classification of DR can be established by the occurrence of neovascularization (NV). Pathological angiogenesis is the hallmark of proliferative DR (PDR) (6), and its absence indicates non-proliferative DR (NPDR) (*Figure 1A-1F*) (7,8). NPDR begins with microangiopathy that consists of pericyte loss with endothelial cell damage, increased
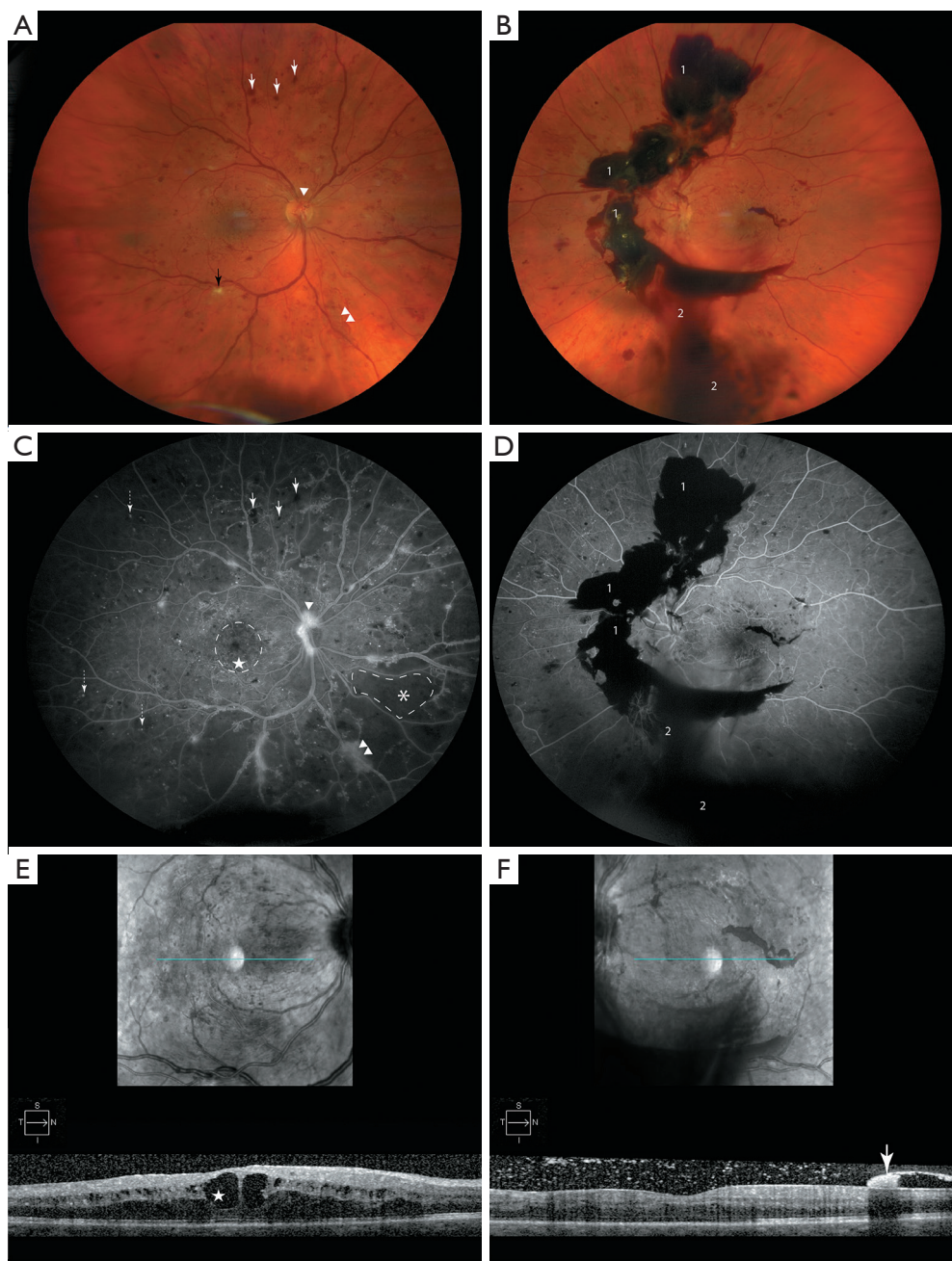
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Figure 1 Features of DR. (A) Color fundus photo of the right eye of a patient with PDR, highlight pathologic changes which include ischemic damage to nerve fibers known as "cotton wool" spot (black arrow), dot-blot hemorrhages (white arrows), NVD (arrowhead) and NVE (double arrowhead). (B) The left eye of the same patient with PDR denotes advanced disease progression, highlighted are pathologic features including pre-retinal/sub-hyaloid bleeding [1] and VH [2]. (C,D) Corresponding FFA images. Pathological features shown here include MAs (dashed arrow) with corresponding features previously mentioned (A,B) and respectively denoted. Petaloid pattern of CME is encircled with dashed line. Area of capillary dropout is marked with asterix (*) and demarcated with dashed line. (E) OCT image of right eye shows cystoid macular edema CME that is more prominent in central macula (star). (F) OCT image of left eye. Hyper-reflective lesion (white arrow) representing pre-retinal/sub-hyoid hemorrhage. DR, diabetic retinopathy; PDR, proliferative diabetic retinopathy; NVD, neovascularization of the disc; NVE, neovascularization elsewhere; FFA, fundus fluorescein angiogram; MA, microaneurysm; VH, vitreous hemorrhage; CME, cystoid macular edema; OCT, optical coherence tomography.

vascular permeability of the blood retinal barrier (BRB), diabetic macular edema (DME), microaneurysms (MAs), dot blot hemorrhages (DBHs) and cotton-wool spots (CWS) (*Figure 1A,1C,1E*) (5,8). NPDR may progress to PDR, with increasing incidence with disease duration. Vision-threatening complications of untreated PDR are vitreous hemorrhage (VH) (*Figure 1B,1D,1F*) and tractional retinal detachment (TRD) that require surgical intervention (8).

Epidemiologic data from 2008–2018 have shown that DR is a growing public health matter, with significant adverse impacts on patients and the health system (9). The therapeutic windows for DR lie in different stages of disease progression. A major focus of ocular therapeutics is aimed at treating DME and NV. The milestone advancement was the discovery of the clinical indications of anti-vascular endothelial growth factors (VEGF) in DR (10-12). Anti-VEGF and corticosteroids have remained the mainstay treatment for many complications of DR. However, a sizeable proportion of patients (~40%) possesses DME resistant to these mono-therapies and a small percentage remain resistant (~8%) to dual therapy leading to diminished visual outcomes (13-16). Therefore, there is an unmet need for new therapeutics to treat vision-threatening sequelae of DR and reverse the course of the disease. Animal models provide means to investigate DR and develop the next generation of ocular therapeutics. In this review, we describe current DR models and outline their benefits and shortcomings in studying different stages of DR and development of novel therapeutics.

Complex diseases such as DM with multifaceted comorbidities are influenced by numerous factors that entail genetics, environmental factors, and chemical exposures (17-23). Advancement of investigational genome-wide technology has propelled the identification of genetic variants associated with DR progression (18). These variants, along with many others, can be induced or genetically engineered in animal models. Gene editing tools such as clustered regularly interspaced short palindromic repeats (CRISPR) and targeted RNA interference (RNAi) technologies allow for precise gene modifications (24,25). In addition to these major technological advancements, selectively breeding animals of desired genetic backgrounds remains as a conventional method for producing genetic animal models.

Inducible disease models can be generated with a wide variety of approaches that include diet modification, drug administration, and surgical procedures (26-29). Selection of animal species has its considerations as there is a sizeable collection to generate DR models for each stage of DR.

Animals used to model DR include rats, mice, canines, feline, swine, rabbit, non-human primates, zebra fish (23).

Of animals used to model DR, the most widely selected are rodents as they provide low cost, short life span, and quick breeding rates; indeed, mice and rats can readily be engineered to exhibit a propensity towards DR pathogenesis (23,30-35). Canines with DR phenotypes have been reported to closely model human DR. However, ethical concerns and cost efficiency impedes widespread use (23,35-40). Felines can be induced to develop DR by altering oxygenation with models generated as early as 1950s (41,42). Recently there has been a growing interest for the use of non-human primates for drug discovery in DR, although previously non-human primates have demonstrated resistance to development of PDR (43,44). Zebrafish, surprisingly, have similar eye structure to humans and are growing in use for their efficient short life span and minimal cost (35,45-48). Although there is a wide range of animal models to study each stage of DR, there is no single model that fully encompasses the entire DR pathogenesis of the human eye.

Rodent models

Rodent models are the most frequently utilized DR animal models. There are several methodologies available to induce various stages of DR, that range from pharmacologic induction, genetic manipulation, environmental exposures, and surgical procedures (23,49-70).

Induction of pharmacologic DR is classically performed by administration of alloxan (a toxic glucose analogue) or the antibiotic streptozotocin (STZ) via destruction of the pancreas seen in type 1 DM (T1DM). Of these two, STZ is the preferred drug for inducing DR, because of the faster rate of phenotype manifestation (52). However, animals in the STZ model rarely develop PDR.

Genetic mouse models include five main genotypes: Ins2Akita (Ins2^{Akita}), nonobese diabetic (NOD), *db/db* (Lepr^{db}), Kimba, and Akimba. Rat specific models focus around six genotypes: Zucker Diabetic Fatty (ZDF), Otsuka Long-Evans Tokushima fatty (OLETF), biobreeding (BB), Wistar Bonn/Kobori (*WBN/Kob*), spontaneously diabetic Torii (SDT), and Goto-Kakizaki (GK). Each genetic model varies in the mode of etiology, inheritance, pathology, and disease progression (64-66,68,70-76).

Environmental induction includes diet and oxygen exposure during early stages of life. A surgical procedure able to induce DR is pancreatectomy. Each model possesses

careful considerations when selecting stage of disease, time to presenting phenotype, costs, and pathology limitations.

Pharmacologically induced rodent models

Dunn and McLetchie performed the first pharmacologic induction of DM in 1942 with administration of the uric acid derivative alloxan (50). The mechanism of DM induced by alloxan is by direct targeting of insulin producing β cells of the pancreas through inhibition of the glucose-insulin pathway enzyme, glucokinase (51). The alloxan induced PDR mouse phenotype manifests between 2–9 months after induction at the age of 8–10 weeks (77). A major limitation of alloxan is the toxic effects on the liver and kidney, although weight adjusted dosing could avoid these adverse effects. Additionally, instability of alloxan at room temperature is a notable issue, and makes handling and administration challenging (50). Due to its side effects, there has been a gradual shift toward the use of STZ.

Initial reports of STZ administration causing DM in rats and dogs date back to 1963 (52). STZ is an antibiotic that was investigated for its use as a chemotherapy and later discovered to induce hyperglycemia with characteristic symptoms of DM. Induction of DM by STZ is by targeting of pancreatic islet with the destruction of β cells via DNA fragmentation facilitated cell death (52). β cells take up STZ via the low affinity glucose transporter 2 (GLUT2), as STZ chemical structure closely resembles that of glucose and N-acetyl glucosamine. As compared to alloxan, STZ shows less nephro- and hepatotoxicity, and it is favored over alloxan for its ease of handling. STZ closely mimics the pathogenesis of DM with induction of hyperglycemia within 2 weeks after induction (35).

Both rats and mice readily develop diabetes with STZ treatment. Surprisingly, rats require lower doses of STZ to exhibit pathologic hyperglycemia (23,35,69). STZ-induced DR typically manifests after long term exposure to high glucose levels greater than 150 mg/dL. Both mouse and rats with STZ DR are widely used to screen compounds for treatment of early stages of T1DM (69), and stem cell therapy (78). Mice with STZ-induced DR exhibit ganglion cell proliferation beginning 4 weeks after onset of hyperglycemia (79). At 16 weeks, the ocular phenotype resembles NPDR seen in humans, with characteristic vascular changes, and thinning of the INL and ONL (80,81). Although rare, the presence of NV has also been reported (82). In rats, STZ-induced DR begins with compromised BRB structural integrity by 2 weeks of diabetes onset, followed ONL thinning at 4 weeks, NV

occurring at 8 weeks, and basement membrane thickening at 1 year (23,69,83). Pharmacologically induced DR models are reliable and straightforward for study designs. The limitations for pharmacologic DR rodent models are mainly related to the adverse effects of the drugs and constraint of disease progression.

Diet-induced rodent models

Diet induced diabetes in rodent models is classically conducted by high-galactose rich diets (27). C57BL/6J mice on high-fat diets (45% fat, 35% carbohydrate, 20% protein) have also shown propensity to develop type 2 DM (T2DM) and provide a model to investigate diet induced DR (27,84). DR phenotypes induced by high-fat diets in mice develop from a similar etiology seen in humans, where DM is a manifestation of high-caloric western diets. DR changes due to hyperglycemia are observed after 21 months with formation of MAs and capillary basement membrane thickening in 30% of mice (7,27,85,86). Rodents with diet-induced DR are commonly used; however, they do not exhibit the exact microvascular damage seen in humans and often lack the proliferative NV (27). As a result, to achieve a good size study group with microvascular manifestations, a large starting sample size as well as extended period of hyperglycemia is required.

Genetic rodent models

Genetic factors play a vital role in the development of DR (87). The majority of rodent genetic models have well characterized genetic backgrounds and are readily manipulated with genetic tools to generate knockouts, knockdowns, and transgenic hybrids. There are five main mouse (Ins2[Akita], NOD, Lepr[db], Kimba, and Akimba) and six main rat (ZDF, OLETF, BB, WBN/Kob, SDT, and GK) specific genetic models of DR. The limitations and advantages vary greatly among genetic models. The following will briefly explain each rodent model and describe similarities in disease pathogenesis.

T1DM models

A missense mutation in *insulin 2* gene causes hyperglycemia via a T1DM-like mechanism. Accumulation of misfolded insulin in β-cells defines the Ins2[Akita] mouse phenotype and leads to apoptosis (64,66). The Ins2[Akita] model is typically used to study T1DM with destruction of β-cells present at 8 weeks. Retinal vascular disease is prominent for up to

36 weeks, with increased permeability and inflammatory damage to the BRB, marked reduction in retinal ganglion cells, and continued damage to both the inner nuclear layer (INL) and inner plexiform layer (IPL) (23,88,89). Limitations of Ins2[Akita] model include its lack of similarity with the vascular changes seen in human DR. These limitations must be considered when using Ins2[Akita] model, as conclusions drawn may require additional supporting evidence to assert correlation (90). The major advantage of the Ins2[Akita] model is its utility for early progression and detection of retinal glial cell damage seen in DR, which provides a useful screening tool for treatments that involve neuroprotection (7). One such treatment involves pigment epithelium-derived factor (PEDF), hypothesized to be neuroprotective via modulation of glutamate transporter expression (91,92). The Ins2[Akita] model is an ideal candidate for PEDF studies or similar treatment investigations.

An alternative to the Ins2[Akita] model is the NOD model, in which mice characteristically develop a T1DM hyperglycemia via an autoimmune response in which CD4+ and CD8+ cells target pancreatic β-cells (23,30,65,93). This model was first discovered in 1974 by Yoshihiro Tochino, who initially observed a female mouse spontaneously developed hyperglycosuria (93,94). Diabetes in NOD mice shows phenotypic resemblance to humans, exhibiting a polygenic etiology with numerous genetic loci correlated to T1DM phenotype (94-97). The NOD model develops DM at 12 weeks and retinal vasculature damage beginning with signs of apoptosis of endothelial cells and retinal glial cells. DR changes, including thickening of the retinal capillary basement membrane begin after 1 month of hyperglycemia (98). Vasoconstriction and degeneration of major blood vessels with compensatory NV manifest by 4 months of hyperglycemia (23,57,99,100). Vitreal injections of pro-inflammatory cytokines (IL-1β and TNF-α) in NOD leads to enhanced disease progression, providing a more directed model to study DR (101,102). Limitations of the NOD model includes a significant gender bias where DM develops by 30 weeks in 80% of female and only 20% of male mice (30). The gender bias in the NOD model may be influenced by gender differences in gut microbiome (103). Underlying gender bias also infers major differences in polygenic background that should be a consideration in data interpretation. Nevertheless, the pathophysiology of T1DM in the NOD model closely follows that observed in humans and provides an excellent mouse model for studies. The NOD mouse model could be used to test compounds aimed at attenuating autoimmune responses or beta islet transplants (104).

Another rodent model frequently used to study T1DM is the biobreeding (BB) rat model, in which an autoimmune mediated apoptosis of pancreatic β-cells causes diabetes. A frameshift mutation found in *immune-associated nucleotide-binding protein* gene (Ian4) is one genetic factor for triggering of autoimmune disease in this model. Additionally, this phenotype produces diabetes associated lymphopenia (105,106). Within 1-year BB rats typically present with pericyte dysfunction, microvascular degeneration, and MAs (75,107). Gut microbiome of BB rats can contribute to the spontaneous T1DM phenotype (108). Further investigation of the exact mechanism of spontaneous T1DM poses a potential limitation of the BB rat model. However, the BB rat model still provides a strong T1DM phenotype for investigations.

T2DM models
The obesity epidemic in the United States has driven the need for research animal models (109,110). T2DM is frequently associated with obesity (111). Two commonly used obesity rodent models include the ZDF rat model and the Lepr[db] mouse model that hold mutations in the *leptin receptor* gene leading to obesity and hyperglycemia (33,112,113).

ZDF rats display a reduction in leptin receptor expression that promotes obesity and serve as an excellent model for research of associated metabolic abnormalities (114). However, these rats may not be an ideal DR model, as retinal vascular damage is often absent even up to 42 weeks of chronic hyperglycemia (114).

Lepr[db] mice carry a mutation in the leptin receptor that results in defective leptin signaling. Homozygous animals become insensitive to leptin, leading to development of obesity and chronic hyperglycemia within the first 2 months of age (112,115). The pathophysiology of diabetes in Lepr[db] mouse model closely mimics that seen in T2DM, with late stages leading to atrophy of pancreatic β cells and dependence on exogenous insulin (112,115). In the retina of Lepr[db] mice, damage to retinal glia cells and increased central retinal membrane thickness is detected after 6 weeks of hyperglycemia (110,116). Characteristics of Lepr[db] mouse include thinning of the ONL seen by 8 weeks and thinning of the INL by 14 weeks of hyperglycemia (116,117). Acellular capillaries and retinal NV present by 18 weeks (118). Limitations of the Lepr[db] mouse model are related to the rapid continuation of severe disease with increasing blood sugar levels and worsening of cardiovascular disease that shortens lifespan to about 10 months (119,120). The primary advantage of the Lepr[db]

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mouse is it provides an exceptional model for screening of late stage DR treatments designed to alleviate the DR symptoms of reactive gliosis and severe vascular damage.

The OLETF rat model spontaneously develops T2DM via a mutation in *G-protein-coupled receptor 10* (*GPR10*) that leads to obesity (72,73). Onset of diabetes is present by 6 months, with abrupt increases in blood glucose (73). Microvasculature changes manifest approximately 6 weeks following diabetes onset with inflammation throughout retinal microcirculation (34). Vascular leakage is noted by 3 months of hyperglycemia, due to damaged endothelial cells and pericyte loss (121,122). Limitations of this model are delayed onset diabetes and absence of a common feature of DR pathology, acellular capillaries.

Another rodent model for studying T2DM is the WBN/Kob rat model that spontaneously develops hyperglycemia via an unknown mechanism regulated by sex hormones (7,123). The causal genes have not been elucidated, but reports suggest regions of significance on chromosome 7 in *WBN/Kob locus 1* (*pdwk1*) (124,125). Retinal disease in this model is marked by intraretinal angiopathy consisting of NV and hyalinization. Although the genotype is unknown, the phenotype elicited is this model still provides a viable research model for understanding DR progression.

Additional rat models are those that develop nonobese T2DM, the SDT and the GK models. Insulin insensitivity is the primary mechanism by which T2DM develops, with atrophy of pancreatic β-cells in late disease. Out of these two rat models, the SDT rat more closely resembles the pathophysiology seen in humans. The genes associated with the SDT model are correlated to three primary genes: *Gisdt1*, *Gisdt2*, and *Gisdt3*, on chromosome 1, 2 and X, respectively; all are related to glucose intolerance (76,126,127). SDT rats develop glycosuria between 20–45 weeks depending on gender (126). Diabetes manifests in male rats by 40 weeks with complete conversion at 65 weeks; however, only 33% of female rats go on to develop disease (126).

SDT rats exhibit retinal disease that features NV without underlying ischemia, as well as apoptosis in both the ganglion cell layer (GCL) and the INL of the retina. Other ocular features of SDT rat include retinal detachment and fibrous remodeling (128), and large retinal folds with extensive vascular leakage surrounds the optic disk, which closely resembles human disease (129-131). A transgenic hybrid, SDT(fa), generated by introduction of the *fatty* (*fa*) allele from the ZDF rat model into SDT rats, displays a more rapid onset of DR (132). One limitation of the SDT model is the lack of MA or NV; therefore, the SDT model is better suited for NPDR research. Similar to the Ins2[Akita] mouse model, SDT model possess a prominent gender bias in onset of disease that is a major consideration for study designs. The major benefit of the SDT rat model is the development of TRD in the late stages of DR.

The GK rat model develops diabetes earlier than SDT model with presentation of hyperglycemia within 4 weeks and DM by 6 weeks (133,134). There is a polygenic background contributing to the GK disease phenotype with reports of 192 potential genes (135). Disease manifestations of GK are defined by reduced retinal blood flow with absence of diameter variation of veins and arteries (7,134). The GK rat model exhibits microcirculatory changes in the retina and loss of pericytes that leads to BRB permeability increase at 3 months following disease onset. This is a good model to study circulatory changes in DR, but it does not show a clear genotype-to-phenotype linkage.

Non-diabetic/hybrid pathology models
Several genetic murine models have been developed with the aim of generating models physiologically similar to human retinal disease. An example is the non-diabetic Kimba mouse, that is genetically modified to overexpress retinal VEGF; driven by mutations at the rhodopsin promoter loci (67,136). The Kimba mouse model manifests retinal disease within the first week of life with markedly reduced ONL and INL. Abnormalities of retinal microvascular are noted by 1 month and pericyte loss by 2 months (136). An additional mouse line, Akimba, was generated by cross breeding Ins2[Akita] mice and Kimba mice (68). The hybrid Akimba mouse, as expected, possesses characteristics from both parental strains that includes hyperglycemia with significant edema and NV (68). A limitation of the Kimba mouse model is early disease development and rapid progression that is present at birth and is focused on the late stage of disease without hyperglycemic etiology. The research benefits of Kimba mice are that DR pathogenesis is controlled by VEGF production, providing an efficient model for treatment development and research focused on NV. Akimba mice are an excellent model for DR research with few limitations common to all animal models. The characteristics from the parental strains are additive and as a result significantly bias retinal disease progression (68).

Environmental exposure rodent models

Oxygen-induced retinopathy (OIR)
Anomalous NV in the posterior segment of the eye is the

hallmark of late stage DR and the distinguishing feature of PDR from NPDR (8,23). The pathogenesis of PDR is driven by chronic hyperglycemia that ultimately leads to vascular damage, and subsequent retinal ischemia that promotes NV. Cycling of hyperoxia and hypoxia produces OIR that physiologically mimics ischemic events and induce compensatory NV. Rodent models of OIR are used to study vascular changes in retinal diseases such as DR (137-139). In OIR models, after exposure to cycling hyperoxia, rodents exhibit two phases of vascular retinal disease. Initially, vasculature regresses in hyperoxic conditions; ensuing ischemia leads to compensatory hypoxia-induced NV (59,137). This hypoxia results in release of angiogenic factors such as VEGF that stimulate angiogenesis in avascular areas to reproduce DR vascular disease (58,140).

Mouse OIR model

The mouse OIR model was first reported by Smith *et al*. in 1994 (58). Commonly used to model retinopathy of prematurity (ROP) but pathologically includes similar features of DR. In it, OIR is generated by exposing neonatal mice to an environment of 75% oxygen at 7 days of age for a duration of at least 5 days, followed by a return to room air for 5 days. The retinopathy begins under hyperoxia with regression of retinal blood vessels from the central zone resulting in vaso-obliteration (138). Exposure to normoxic conditions leads to ischemia and release of hypoxic-inducible factors, such as erythropoietin and VEGF that trigger angiogenesis (58,60,140). Gradual vascularization of the vaso-obliterated areas mimics human proliferative retinopathies such as DR and ROP (58,141). Disease features of the OIR mouse model include reduction in INL and IPL thickness and degeneration of BRB with increased vascular leakage. New blood vessels are formed distinctly and termed "endothelial tufts" that may protrude into the vitreous (58,141). The OIR mouse model provides an excellent example to study PDR, because the pathologic angiogenesis resembles human disease. Studies using the OIR mouse model demonstrate that the inhibition of VEGF signaling via soluble VEGF receptor 1 antibody reduces pathologic retinal NV (63). Major limitation of this model are the absence of chronic hyperglycemia, thus limiting the studies to microvasculature changes seen in PDR and retinal NV which is transient and spontaneously regress, unlike PDR.

Canine DR models

Surgical procedures were the primary method used to induce diabetes in early animal models. Historically insulin was first isolated from pancreas of canines for treatment of "Juvenile Diabetes" (49), an immune mediated T1DM. Canines develop diabetes shortly after pancreatectomy and provided one of the earliest animal models to study diabetes (7,29). In addition to pancreatectomy, other methodologies used to generate canine DR models include high-galactose diet induction, alloxan/STZ administration, and growth hormone regimens (7). Canines on long-term high galactose diets develop DR, with severe stages of disease manifesting by 5 years (37,142,143). Morphology of DR in canines closely resembles disease seen in humans (144), and includes many of the classical features such as MAs, pericyte loss, thickened capillary basement membrane, and dot and blot hemorrhages (145). Pathologic vascularization is often absent, limiting this model to NPDR studies. The high cost of canine care and the ethical unpopularity of using canines in research have impeded the use of canine models for DR research studies.

Feline models

Felines can develop DM and display the same pathological characteristics as human T1DM and T2DM (144,146). Pancreatectomy is often used to induce glucose intolerance. However, the time to feline DR phenotype ranges from 5 to 9 years of chronic hyperglycemia (146,147). Felines can display retinal changes including CWS, vascular leakage, and NV (147,148). MAs in felines have been reported in some studies and absent in others (148). Alloxan and STZ are also effective modalities in developing DR in felines and often used as complements to partial pancreatectomy (149). The feline OIR model was first described in the 1950s, as one of the earliest models exploring the effects of oxygen concentration on retinal disease (41). Similar to the rodent OIR model, kittens were exposed to hyperoxic conditions (80% oxygen) for 4–5 days, which induced retinal vessel regression and vaso-obliteration. When kittens are returned to room air (21% oxygen), hypoxia-induced angiogenesis develops (41,42). The feline OIR model exhibits similar disease features to mouse OIR model. However, felines do not display retinal detachment. There are multiple limitations with using feline models, including long incubations required for DR development and unavailability of research reagents. These limitations make this model difficult to use. However, felines exhibit a moderate resistance to DR and may offer a novel platform to investigate severe stages of disease in future studies.

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Swine models

Swines have found utility in many disciplines of disease research. The human eye and the swine eye share similarity in size, retinal structure, and vasculature; these are appealing characteristics for modeling DR (150). The majority of swine eye research is not performed with *in vivo* models, but rather using *in vitro* experiments with swine retinal cells, specifically Müller cells (151,152). Swine *in vivo* DR models can be pharmacologically induced by administration of alloxan or STZ (151), and biologically induced with intravitreal injections of retinal pigment epithelial (RPE) cells (153). Alloxan-induced DR in swine exhibit retinal basement membrane thickening, pericyte destruction, and vascular edema within 4–6 months of hyperglycemia (154). Transgenic swine models also demonstrate signs of DR. Swine carrying a mutated human *HNF-1α* (P291fsinsC) developed chronic hyperglycemia (>200 mg/dL) by 1 month of age. NPDR features such as retinal hemorrhage and CWS were detected by 4 months of hyperglycemia, yet PDR was not observed in this model. This limited study requires further investigation (155). Development of swine DR models may provide an excellent means for therapeutic development that may overshadow the cost limitations and scarcity of research reagents.

Rabbit models

Rabbit models of DR have been generated by pharmacologic agents, altered diet, or direct implantations of VEGF (inherently non-diabetic) into the retina. Each has its own drawbacks. Pharmacologic induction is the most common method to induce DR in rabbits. Typical agent use is STZ (100 mg/kg), which abruptly increases blood glucose levels (>200 mg/dL) (156). DR lesions present with variable distribution within 5 months of STZ administration. Approximately 50% of rabbits develop PDR. Interestingly, 40% only show MAs, DBH, and thrombosis. The remaining 10% exhibit only hard exudates and moderate microangiopathy (35,156).

Diet-induced DR in rabbits is achieved with high-caloric-hyperlipidemic diet (157). However, minimal DR development and long incubation time make this approach less attractive.

Direct implantation of VEGF is a viable method to induce DR changes in rabbits (158-160). Rabbits develop PDR with NV within 3 weeks of VEGF polymeric implant (160). However, regression of NV occurs 5 weeks after implantation leaving a short window for study. The use of human recombinant basic fibroblast growth factor (bFGF) in a polymeric implant yields more efficient DR development within 1–2 weeks of implantations and DR features are hemorrhage, NV, and TRD (158).

The rabbit model provides an excellent means to screen therapeutic compounds, as the DR phenotype can be achieved in a short time, and the resultant retinal pathology is similar to human disease. The limitations of this model are high cost and variation of DR among rabbit species (159).

Non-human primate models

DM may be elicited in non-human primates by chemical or surgical induction (44,161,162). Despite the presence of long-term hyperglycemia in these models, non-human primates are particularly resistant to PDR (44). After administration of Alloxan or STZ, a long duration of hyperglycemia is required before early sings of DR are detected (44,161). This resilience suggests that non-human primates possess means to minimize the effects of widely uncontrolled chronic hyperglycemia.

Cynomolgus macaque or obese rhesus monkeys display spontaneous T2DM, with retinal ischemia, MAs, CWS, intra-retinal hemorrhages and hard exudates in the macula without NV (43,161). One of the major limitations of non-human primates for drug discovery is the long housing duration before DR develops (43). Due to lack of NV, non-human primates are limited to NPDR studies (7). The major benefit that non-human primate models provide is the presence of a macula that is not found in other animal models. Future directions for developing DR models of non-human primate require a better understanding of biological mechanisms that play a role in NV during chronic hyperglycemia.

Zebrafish models

A unique DR animal model that surprisingly shares similar eye structure to the human eye is zebrafish. DR can be generated in adult zebrafish with hypoxia induction (163,164). Transgenic fluorescent zebrafish Tg(fli1:EGFP)y that are exposed to hypoxic conditions with ~10% air for 12 days develop new blood vessels that are readily identifiable with GFP imaging (165). Genetic mutation of the *von Hippel-Lindau tumor suppressor* gene (*vhl1*) also results in a DR-like angiogenesis (166). Zebrafish carrying this *vhl1* mutation have pronounced retinal vascular formation and upregulation

of VEGF. DR features in this model include increased number of hyaloid vasculatures with simultaneous vascular leakage, retinal edema, severe NV and ensuing retinal detachment (166). A major advantage of using zebrafish is its ease of genetic manipulation, in addition to its low cost and quick breeding rate. Zebrafish serve as a viable DR model that requires further characterization and can expedite empiric drug screenings exponentially (45).

Conclusions

The prevalence of diabetes and its complications continue to remain a major public health concern in the United States. The utilization of animal models serves a vital role by furthering the understanding of DR pathophysiology, progression, and etiology. Future advancement of DR animal models is required as DR is a multifaceted disease including vascular and neurologic components that are influenced by both genetics and environmental exposures. While no single animal model encompassing the entire DR pathogenesis exists, we find promise in furthering comprehension of genetic models and development of new high-order animal models. To this end, development of ocular therapeutics for DR treatments holds a favorable future.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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