Chapter 3

Animal Models of Diabetic Retinopathy (Part 2)

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Additional information is available at the end of the chapter

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Abstract

Diabetic retinopathy (DR) is one of the leading causes of preventable vision impairment and blindness in the working-age population worldwide. Numerous animal models have been developed for therapeutic drug screening and to further increase our understanding of the molecular and cellular pathological processes involved in DR. Following our discussion of mouse models in "Animal Models of Diabetic Retinopathy Part 1," we describe the cellular, molecular, and morphological features of both rodent and nonrodent models of DR and their respective advantages and limitations in this chapter. To date, no animal model can holistically reproduce the pathological progression of human DR; most only display early or advanced lesions of DR. However, a thorough understanding of genotypic and phenotypic expressions of existing models will facilitate researchers' selection of the appropriate model to simulate their desired clinical scenarios.

Keywords: animals, blood glucose, blindness, diabetic complications, diabetes mellitus/pathology/physiopathology, neovascularization, proliferative, retinal vessels

1. DR features of animal models

Among all the existing animal models of diabetic retinopathy (DR), mice and rats are most commonly used, possibly due to their small size, availability, genetic tractability, and relatively faster development of DR lesions as compared with larger animals. **Tables 1–3** summarize the cellular, molecular, and morphological features of rat and nonrodent models of DR. Features of mouse models are detailed in the previous chapter (Animal Models of Diabetic Retinopathy Part 1).

Rat model	Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in rat models (Age at which correlates are first reported unless otherwise specified) (⁺Time post treatment: diabetes, galactosemia, or induction of VEGF overexpression)		
			NPDR features	**PDR features**	**Functional changes (ERG)**
STZ injection	1	Within one wk	• *1 wk*⁺: reduced total retinal thickness [93] • *2 wks*⁺: increased retinal apoptosis [94] BRB breakdown [5, 93] (*16 wks*⁺ [6]) • *4 wks*⁺: Müller cell gliosis [2, 3] (*6 wks*⁺ [4]) (*12 wks*⁺ [5]) Reduced NeuN-positive cells in GCL [3] (*4 mo* reduced number of cells in GCL [1]) Reduced astrocyte number [5] (*6 wks*⁺ [2]) Increased number of microglia [5] (*4 mo*⁺ [3]) Reduced number of cells in ONL [93] • *6 wks*⁺: reduced astrocyte processes [2] • *8 wks*⁺: increased adherent leukocytes [7] • *16 wks*⁺: increased acellular capillaries [6] (*8 mo*⁺ [1]) Decreased number of pericytes [6] • *4 mo*⁺: reduced NeuN-positive cells in INL [3] • *8 mo*⁺: pericyte ghosts [1] • *12 mo*⁺: capillary BM thickening [8]		• *2 wks*⁺: decreased b-wave amplitude [4] (*11 wks*⁺ [9]) (*16 wks*⁺ [6]) Decreased OP amplitude [4] (*16 wks*⁺ [6]) • *8 wks*⁺: decreased OPs (not observed at 11 wks) [95] • *11 wks*⁺: increased a-wave implicit time [9] Decreased a-wave amplitude [9] (*10 wks*⁺ [4]) Delayed OPs [9]
Alloxan injection	1		• *6 wks*⁺: BRB breakdown [96] • *5 mo*⁺: capillary occlusion with endothelial cell swelling [11] • *8 mo*⁺: retinal microvascular cell death [10] • *9 mo*⁺: IRMAs in lower capillary layer [11] • *12 mo*⁺: pericyte ghosts [12] Increased no. of acellular capillaries [12] (*18 mo*⁺ [10]) Capillary BM thickening [12]	• *2 mo*⁺: neovascularization in mid-periphery region [11] • *5 mo*⁺: neovascularization in center and mid-periphery regions [11] • *9 mo*⁺: neovascularization in all regions [11]	

Rat model	Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in rat models (Age at which correlates are first reported unless otherwise specified) (⁺Time post treatment: diabetes, galactosemia, or induction of VEGF overexpression)		
			NPDR features	**PDR features**	**Functional changes (ERG)**
Galactose-fed	/	/	• *4 mo⁺*: increased retinal microvascular cell apoptosis^ [10] • *12 mo⁺*: pericyte ghosts [12] (*23 mo⁺*)*^ [13] Increased no. of acellular capillaries [12] (*23 mo⁺*)*^ [13] Capillary BM thickening [12] (*23 mo⁺*)*^ [13] (*28 mo⁺*)^ [14] • *24 mo⁺*: dilated, hypercellular vessels*^ [13] IRMAs* [13] • *28 mo⁺*: disrupted retinal layers^ [14] Gliosis^ [14] Capillary dilatation^ [14] Microaneurysms (OPL, INL)^ [14] Increased number of endothelial cells^ [14] **50% galactose diet; ^30% galactose diet*		
BB rat	1	60–120 days (90% hyperglycemic by 90–120 days) [15]	• *6 mo*: increased retinal capillary BM thickness [15, 18] • *7.7 ± 1.1 mo*: reduced and deranged basal infoldings of the RPE basal plasmalemma [16] • *8 mo⁺*: decreased pericyte/endothelial cell ratio [17] Decreased number of pericytes [17] Microinfarctions with nonperfused areas [19]		

Rat model	Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in rat models (Age at which correlates are first reported unless otherwise specified) (*Time post treatment: diabetes, galactosemia, or induction of VEGF overexpression)		
			NPDR features	**PDR features**	**Functional changes (ERG)**
WBN/ Kob rat	2	9–12 months of age [20]	• *5 mo*: decreased outer segment thickness [21] • *10 mo*: clustering of capillaries into tortuous knots [22] • *11 mo*: decreased OPL and ONL thickness [21] • *14 mo*: capillary BM thickening [21] Complete photoreceptor cell nuclei and ONL loss ([21, 22], 169) • *15 mo*: decreased number of capillaries [22] Capillary loop formation [22] • *19 mo*: acellular capillaries [24]	*24 mo*: • Intravitreal and choroidal neovascularization [23, 24] • Increased hyalinization of intraretinal vessels [23] • Increased intravitreal proliferation of fibrovascular elements [23]	
ZDF rat	2	6–7 wks of age [25]	• *14 wks*: increased VEGF-beta mRNA levels in retina [26] • *27 wks*: capillary BM thickening [28] (6–7 mo) [27] Increased capillary hypercellularity [28] (6–7 mo) [27] • *33–34 wks*: increased pericyte apoptosis and presence of pericyte ghosts [29] Increased endothelial cell apopotosis [29] Increased number of acellular capillaries [29]		

Rat model	Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in rat models (Age at which correlates are first reported unless otherwise specified) (†Time post treatment: diabetes, galactosemia, or induction of VEGF overexpression)		
			NPDR features	**PDR features**	**Functional changes (ERG)**
OLEFT	2	18 wks of age [30]	• *24 wks*: increased leukocyte entrapment [38] • *28 wks*: reduced total retinal and RNFL thickness [32] • *36 wks*: reduced number of RGCs [32] Increased apoptosis in RNFL [32] • *40 wks*: increased GFAP immunoreactivity in Müller cells [33] • *14 mo*: capillary BM thickening [31, 34] Endothelial cell degeneration [31, 34] Reduced pericyte area to total capillary cross-sectional area ratio [34] Caliber irregularity [34] (*64 wks*) [35] Capillary tortuosity [34] (*64 wks*) [35] (*17 mo*) [31] Capillary loop formation [34] (*64 wks*) [35] (*17 mo*) [31] • *64 wks*: capillary narrowing [35] Microaneurysms [35] (*17 mo*) [31] • *19 mo*: decreased INL and photoreceptor thickness [31] Decreased RPE height [31] Poorly developed basal infoldings [31]		*60 wks*: • Prolonged peak latencies of OPs [36]

Rat model	Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in rat models (Age at which correlates are first reported unless otherwise specified) (+Time post treatment: diabetes, galactosemia, or induction of VEGF overexpression)		
			NPDR features	**PDR features**	**Functional changes (ERG)**
GK rat	2 (non-obese)	4–6 wks of age [37] (glucose in-tolerance as early as at 2 wks of age)	• *1 mo*+: increased retinal mean circulation time [38] • *3 mo*+: increased BRB permeability [39] • *28 wks*: increased ocular VEGF concentrations [41] • *8 mo*: Increased endothelial/pericyte ratio [40] • *12 mo*+: Activated microglia [42]	*1 mo* (*post hyperglycemia*) • *1 mo*+: reduced retinal segmental blood flow [38] • *12 mo*: migration and accumulation of microglia/ macrophages in the subretinal space [42]	
SDT	2 (non-obese)	25 wks of age (male rats) (100% diabetic by 40 wks of age) [44]	• *40 wks*: increased cellular apoptosis in GCL and INL [45] Increased GFAP immunoreactivity expression across the whole retina [45] • *44 wks*: increased retinal leukostasis [46] • *52–60 wks*: increased INL, ONL, PL, choroidal and total retinal thickness [47] • *68 wks*: fluorescein leakage around the optic disc [48] Venous dilation [48] Distorted retina [48] (*70 wks*) [44] Protrusion of optic disc into the vitreous • *70 wks*: retinal thickening [44] • *77 wks*: anterior chamber hemorrhage with fibrovascular changes around the iris [44, 49] • *49–82 wks*: acellular capillaries [49] Pericyte loss [49] Tortuous vessels [49] Capillary nonperfusion [49] • *20 mo*: increased VEGF immunoreactivity in vascular endothelial cells, GCL, INL, border between ONL and OS [50]	• *70 wks*: tractional retinal detachment with fibrous proliferation [44, 49] • *20 mo*: neovascularization (53%) without ischemia [50] • Tractional changes without retinal detachment [50]	*32 weeks* • Prolonged OP peak latencies [48] *44 weeks* • Reduced a-, b-wave and OP1, OP2, OP3 amplitudes [51] • Increased OP2, OP3, OP4 implicit time [51]

Rat model	Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in rat models (Age at which correlates are first reported unless otherwise specified) (⁺Time post treatment: diabetes, galactosemia, or induction of VEGF overexpression)		
			NPDR features	**PDR features**	**Functional changes (ERG)**
TetO rat [52]	2	7–8 days after incorporation of doxy-cycline in drinking water	*4–5 weeks*⁺ [52] • RGC loss • Reduced number of pericytes • Increased number of stenotic vessels • Increased number of veins with abnormal vessel caliber (deep microvascular plexus) • BRB breakdown • Increased GFAP expression • Reactive gliosis • Increased inflammatory markers	*P18* • Intravitreal neovascularization in peripheral retina [54, 58]	*ERG Changes 4–5 weeks after hyperglycemia onset* • Reduced a-waves, b-waves, and c-wave amplitude • Elevated b/a-wave ratio
OIR	/	/	• *P13*: perivascular astrocyte loss [54] • *P14–16*: increased vascular permeability (ceased at p18) [55] • *P18*: reduced peripheral INL(and central), IPL and total retinal thickness [56] Increased Müller cell gliosis in peripheral retina [54, 56] Increased tortuosity of arterioles [57] Decreased vessel profiles in outer and inner plexuses in peripheral retina [54, 58] Altered pericyte-endothelial interactions [54] Shortened retinal outer segment length [97]		*ERG changes at p18* • Reduced a-wave and b-wave amplitude [57]

This table has been modified from a review by Lai and Lo [19].

Table 1. Summary of the cellular, molecular, and morphological features displayed in rat models of DR.

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Model		Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in non-rodent models (Age at which correlates are first reported unless otherwise specified) (⁺Time posttreatment: diabetes, galactosemia, or induction of VEGF overexpression)		
				NPDR features	**PDR features**	**Functional changes (ERG)**
Dog	Galactose-fed	/	/	• *19 mo*⁺: pericyte loss [62] (*36 mo*⁺: increased endothelial/pericyte ratio [63]) • *24 mo*⁺: uneven distribution of endothelial cells [62] Acellular capillaries [98] (*36 mo*⁺ [63]) Endothelial proliferation (in areas with pericyte loss) [62] (*36 mo*⁺ [63]) • *27 mo*⁺: microaneurysm [62] (*32–36 mo*⁺ [64]) (*36 mo*⁺ [63]) • *33 mo*⁺: dot and blot hemorrhages [62, 64] Capillary varicose enlargement [62] Areas of nonperfusion [99] (*36 mo*⁺ [65] (*56 mo*⁺ [62]) • *36–48 mo*⁺: hemorrhage [62] • *56 mo*⁺: preretinal hemorrhage [62] IRMAs [62] • *60 mo*⁺: AV shunts [62] Arterial node formation [62] Gliosis [62]	• *60 mo*⁺: neovascularization [65] • *84 mo*⁺: intravitreal retinal vascular growth [62] Partial detachment of posterior vitreous [62]	
	STZ injection [60]			• *1–3 yrs.*⁺: capillary BM thickening • *4 yrs.*⁺: pericyte loss Smooth muscle cell loss • *7 yrs.*⁺: microaneurysms Acellular capillaries IRMAs		
	Alloxan injection			• *5 yrs.*⁺: microaneurysm [61] Pericyte ghosts [61] Increased endothelial/pericyte ratio [61] Increased acellular capillaries [61] BM thickening [61] Vascular leakage, increased permeability of retinal blood vessels [100]		

Model		Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in non-rodent models (Age at which correlates are first reported unless otherwise specified) (⁺Time posttreatment: diabetes, galactosemia, or induction of VEGF overexpression)		
				NPDR features	**PDR features**	**Functional changes (ERG)**
Cat	Pancreatotomy	1	1–2 weeks post surgery	• *3–10 mo*⁺: capillary BM thickening [70] • *5 yrs.*⁺: microaneurysm [69] (*7.7–9.4 yrs.*⁺ [71]) • *6.5 yrs.*⁺: small intraretinal hemorrhages [69] (*7.7–9.4 yrs.*⁺ [71]) • *7.5 yrs.*⁺: IRMAs [69] Capillary non-perfusion areas [69] (*9.4 yrs.*⁺ [71]) • *8.5 yrs.*⁺: microvessel dilation and tortuosity	• *8.5 yrs.*⁺: small foci of neovascularization	*9.4 yrs.*⁺: decreased b-wave (trend) [71]
Rabbit	STZ injection	1		*135 days*⁺: • 10% eyes: "Moderate vasculopathy with hard or soft exudates, widespread hemorrhages" [75] • 40% eyes: "Serious vasculopathy with serious retinal and preretinal hemorrhages, vascular lesions, hemovitreous, venous thrombosis" [75]	• *135 days*⁺: 50% of eyes: proliferative retinopathy [75]	
	Diet-induced [72]	2	12–24 weeks	• *12 wks*⁺: increased number of microaneurysms		
	VEGF implant	/	/	• *7 days*⁺: increased dilation and tortuosity of vessels [73] • *14 days*⁺: vascular leakage [73]	• *14 days*⁺: neovascularization (decreased after 21 days with almost complete regression by 35 days) [73] *VEGF + bFGF* • *48 hrs*⁺: grade + 1 neovascularization [74] • *4 days*⁺: grade 4 neovascularization [74] • *2 wks*⁺: total traction retinal detachment with significant hemorrhage [74]	

Model		Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in non-rodent models (Age at which correlates are first reported unless otherwise specified) (⁺Time posttreatment: diabetes, galactosemia, or induction of VEGF overexpression)		
				NPDR features	**PDR features**	**Functional changes (ERG)**
Swine	STZ injection	1	1 wk [79]	• *18 wks*⁺: capillary BM thickening with rarefaction [79]		
	Alloxan injection	1	15–30 days [76]	• *90 days*⁺: increased GFAP in Müller cells [76] • *20 wks*⁺: capillary BM thickening [77] • *20 wks* (*age*): pericyte loss [78] Reduced total number of BRB capillaries [78] Capillary collapse [78]		
Zebra fish	Glucose-induced	1	1–2 days [90]	• *Day 28*: decreased IPL and INL [90]		
	Glucose-induced (*flk*:EGFP transgenic zebrafish)			• *6 dpf* (*3 days of glucose tx*): increased diameter of hyaloid-retinal vessels [89]		
	Hypoxia-induced	/	/	• *3 days*⁺: increased capillary sprouts in arterioles and veins [91] Increased number of branch points [91] Reduced intercapillary distance [91]	*3 days*⁺: neovascularization (increased vascular area) (plateau at day 12) [91]	
	vhl mutants	/	/	• *5.75 dpf*: increased hyaloid and choroidal vascular networks [101] • *6 dpf*: vascular leakage *dpf = days post fertilization*	*7.5 dpf*: • Severe macular edema [101] • Retinal detachment [101] • Intraretinal neovascularization (predominantly in IPL) [101]	

This table has been modified from a review by Lai and Lo [19].

Table 2. Summary of the cellular, molecular, and morphological features displayed in nonrodent models of DR.

Model		Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in non-human primate models (Age at which correlates are first reported unless otherwise specified) (+Time post treatment: diabetes, galactosemia, or VEGF overexpression–induction)		
				NPDR features	**PDR features**	**Functional changes (ERG)**
Monkey	STZ injection, spontaneous or pancreatectomy [81]	1	/	*Changes observed in diabetic monkeys with spontaneous or pharmacologically-induced hypertension* • *6–15 yrs.*+: focal intraretinal capillary leakage spots Capillary dilatation Capillary dropout Cotton wool spots Microaneurysms Arteriolar dilatation and occlusion Atrophic retinopathy		
	Spontaneous, obese	2	/	• *3–8 yrs.*+: cotton wool spots [82] Capillary dropout [82] Microaneurysms Kim, #413} IRMAs [82] Nonperfused areas [82] • *8 yrs.*+: RGC loss [83] Decreased ONL, IS, OS thickness [83]	• *3–8 yrs.*+: cystoid macular edema [82]	*8 yrs.*+: • Reduced OPs [83] • Reduced amplitudes in mfERG • Delayed a-wave [83]
	Intra-vitreal VEGF injection	/	/	• *2 days*+: tortuous vessels [85] Vascular leakage [85] • *3 days*+: vessel dilatation [84] • *18 days*+: microaneurysms [84] Intraretinal hemorrhage [84] Venous beading [84] • *30 days*+: intraretinal hemorrhage and edema [84]	• *2 days*+: grade 2 iris neovascularization [85] • *8 days*+: grade 3 iris neovascularization [85] • *19 days*+: neovascular glaucoma [85]	
	VEGF implant	/	/	• *3 wks*+: vascular leakage (BRB breakdown) [73] • *2–3 wks*+: dilation of retinal vessels [73] *Note*: *vascular changes peaked at 3 wks*	• *1 wk.*+: iris neovascularization (regressed between wks 2–3)	

Model		Type of diabetes	Hyperglycemia onset	Cellular, morphological, and vascular features of human DR displayed in non-human primate models (Age at which correlates are first reported unless otherwise specified) (⁺Time post treatment: diabetes, galactosemia, or VEGF overexpression–induction)		
				NPDR features	**PDR features**	**Functional changes (ERG)**
Marmoset	Galactose-fed (30%) [86]	/	/	• *2.5 yrs.*⁺: microaneurysm Increased acellular capillaries Pericyte loss BM thickening BRB breakdown	• Macular edema and thickening	

This table has been modified from a review by Lai and Lo [19].

Table 3. Summary of the cellular, molecular, and morphological features displayed in nonhuman primate models of DR.

1.1. Rat models

1.1.1. Pharmacological

1.1.1.1. Streptozotocin (STZ) injection

Streptozotocin (STZ) injection diabetic rats reproduce early symptoms of DR, though prominent differences have been identified between rat strains possibly as a result of differing genetic susceptibility to DR development. A report comparing differences in the development of DR lesions in Sprague Dawley, Lewis, and Wistar rats revealed that neuronal loss in the ganglion cell layer (GCL) occurred only in Lewis rats, while both Lewis and Wistar rats showed degeneration of capillaries with pericyte ghosts after 8 months of hyperglycemia [1]. Sprague Dawley rats did not develop any lesions.

In general, neuronal and glial changes appear to precede vascular changes in this model. Retinal apoptosis and retinal thinning developed within 1–2 weeks of hyperglycemia. Müller cell gliosis, as evidenced by increased glial fibrillary acidic protein (GFAP) expression, was prominent beginning at 4 weeks of hyperglycemia [2–5]. Studies have also documented a reduction in astrocyte number and processes [2, 5], decrease in the number of cells in the GCL [1, 3], and an increase in number of microglia [1, 3] after 4–6 weeks of hyperglycemia. Blood-retina barrier (BRB) breakdown was present after 2 weeks of hyperglycemia, while other vascular changes, including acellular capillaries [1, 6], leukostasis [7], and arterial and venous capillary basement membrane (BM) thickening [8], were only present after a longer period of hyperglycemia. Pericyte loss became evident after 4 [6] or 8 months [1] of hyperglycemia. Retinal dysfunction was evidenced by decreased b-wave [4, 6, 9] and OP amplitude [4, 6] and decreased a-wave amplitude [4, 9] with delayed oscillatory potentials (OP) [9] as early as at 2 weeks and 10 weeks, respectively.

1.1.1.2. Alloxan injection

Studies using alloxan injections for diabetes-induction are limited, and none have assessed for neurodegenerative lesions of DR. Alloxan-induced diabetic rats were found to have BRB breakdown within 6 weeks of hyperglycemia. With a longer duration of diabetes, there was retinal microvascular cell death [10], intraretinal microvascular abnormalities (IRMA) [11], pericyte ghosts [12], acellular capillaries [10, 12], and capillary BM thickening [12]. Neovascularization began in the mid-peripheral retina after 2 months of hyperglycemia and extended to all regions of the retina by 9 months [11].

1.1.2. Diet induced

Galactose-fed rats display many retinal microangiopathy features that resemble non-proliferative diabetic retinopathy (NPDR). Four months of 30% galactose-fed diet resulted in increased retinal microvascular cell apoptosis [10]. With a longer duration of galactosemia, pericyte ghosts, acellular capillaries, vessel dilation, capillary BM thickening, and IRMAs became evident in both 30 and 50% galactose-fed rats [12–14]. Long-term galactosemia of 28 months further resulted in gliosis, displacement or disruption of retinal layers, an increase in the number of endothelial cells, and microaneurysm formation in the outer plexiform layer (OPL) and inner nuclear layer (INL) [14].

1.1.3. Transgenic

1.1.3.1. Biobreeding (BB) rats

The diabetes-prone biobreeding (BB) rat is a T1D model derived from outbred Wistar rats. BB rats develop insulitis with selective β-cell destruction, hyperglycemia, hyperketonemia, ketonuria, and hypoinsulinemia. Rapid progression to fatal diabetic ketoacidosis results if exogenous insulin is not administered. The majority of rats develop hyperglycemia by the age of 90–120 days [15]. Existing reports have demonstrated reduced and deranged basal infoldings of the retinal pigment epithelium (RPE) basal plasmalemma [16], pericyte loss, reduced pericyte to endothelial cell ratios [17], capillary BM thickening [15, 18], and microinfarctions [19]. Microaneurysm formation and neovascularization were absent even after 11 months of diabetes [19]. Characterization studies of DR using this model remain limited. Subsequent inbreeding and outbreeding have produced various genetically distinct substrains (named after the origin of the colony), including the BBDP/Wor, BB/OK, and BB/Pfd rat. Care should be taken when comparing results of studies utilizing rats of different substrains due to potential genetic and phenotypic differences.

1.1.3.2. Wistar Bonn/Kobori (WBN/Kob) rats

The Wistar Bonn/Kobori (WBN/Kob) rat is a nonobese T2D strain of Wistar rats that develop exocrine pancreatic insufficiency characterized by α- and β-cell destruction. Male rats develop hyperglycemia at around 9–12 months of age, while females remain unaffected [20].

The retinal changes in this rat model have long been argued to be more degenerative than diabetic. At the age of 5 months, prior to the development of hyperglycemia, the rats showed signs of retinal degeneration with the loss of rods and cones and thinning of the photoreceptor layers [21]. Although retinal capillary changes, such as capillary BM thickening [21], reduced capillary number, formation of capillary loops, presence of acellular capillaries, and clustering of capillaries into tortuous knots [22], were observed after hyperglycemic development, it is uncertain whether these changes arose from the development of diabetes or resulted from further progression of retinal degeneration. WBN/Kob rats survive for a longer duration under diabetic conditions compared to other models. Perhaps, this prolonged survival and hyperglycemia duration had led to the development of intravitreal and choroidal neovascularization, which was accompanied by the hyalinization of intraretinal vessels and increased proliferation of fibrovascular elements in the vitreous at 24 months of age [23, 24]. Such features had not been previously reported in other rats. The proliferative changes were mostly absent in nondiabetic age-matched female counterparts [23], suggesting that the WBN/Kob rat may serve as a diabetic angiopathy model. Nevertheless, the retinal degenerative changes in the WBN/Kob rats are arguably different from that of human DR, making it a less suitable model for DR.

1.1.3.3. Zucker diabetic fatty (ZDF) rats

The Zucker diabetic fatty rat (ZDF/Gmi, fa/fa) is a T2D model derived from the partial inbreeding of the fa/fa line. The rats carry an inherited mutation in the leptin receptor and need to be maintained on a specialized diet to ensure consistent diabetes development. Male rats display marked

hyperglycemia, hyperinsulinemia, insulin resistance, and obesity at 6–7 weeks of age [25]. Studies have reported increased vascular endothelial growth factor (VEGF) expression in the retina that correlated with retinal levels of inflammatory markers at 14 weeks of age [26], capillary BM thickening with increased retinal capillary hypercellularity at 27 weeks [27, 28], and increased pericyte apoptosis, endothelial apoptosis, presence of pericyte ghosts and number of acellular capillaries as compared to the lean controls by 33–34 weeks [29]. The rats have a relatively short lifespan of 1 year but do not require insulin treatment while exhibiting consistently marked hyperglycemia with glucose levels averaging at 500 mg/dL when fed with the appropriate diet. To date, few retinal morphological and therapeutic drug studies have been performed on this rat model.

1.1.3.4. Otsuka Long-Evans Tokushima fatty (OLETF) rats

The Otsuka Long-Evans Tokushima fatty (OLETF) rat is a T2D model originally generated from selective breeding of the Long-Evans rats that spontaneously exhibited polyuria, polydipsia, and obesity. The OLETF rat is characterized by mild obesity, a late onset of chronic hyperglycemia after the age of 18 weeks, male inheritance, pancreatic islet hyperplasia, and the development of T2D as a result of metabolic syndrome [30]. Significant weight gain occurs between the age of 1 and 6 months followed by significant weight loss after the age of 9 months [31].

The rats display an array of retinal cellular morphological changes corresponding to the early stage of DR. Increased leukocyte entrapment was first reported in 24-week-old rats. Spectral domain optical coherence tomography (SD-OCT) revealed significant thinning of the total retinal and retinal nerve fiber layer (RNFL) at the age of 28 weeks [32]. There was retinal ganglion cells (RGC) loss with nonuniform distribution of remaining RGCs and increased apoptosis in the RNFL at 36 weeks [32]. GFAP and VEGF immunoreactivity were upregulated, with VEGF expression extending across all layers of the retina into the OPL [33]. By 14 months of age, there were endothelial cell degeneration, capillary BM thickening, and pericyte loss [31, 34]. A longer duration of hyperglycemia resulted in the reduction in INL thickness, photoreceptor thickness, and RPE height with poorly developed basal infoldings, suggestive of neurodegeneration. Rats aged 14–17 months have consistently exhibited marked microvascular changes, including vessel tortuosity, caliber irregularity, capillary loop formation, capillary narrowing, and microaneurysms [31, 34, 35]. One study further revealed prolonged peak latencies of OPs on ERG, particularly OP1 and OP3, [36], although experimental conditions may have accounted for such abnormalities.

Existing characterization studies have only been performed by a selective number of research groups. Despite the similarities in the pathophysiology of T2D in OLETF rats and humans, the use of OLETF rats in DR studies is limited by the model's late onset of hyperglycemia and absence of late-stage DR features. The presence of hemorrhages, exudates, and acellular capillaries has not been reported in rats up to 19 months of age [31], signifying its unsuitability as an angiopathic DR model.

1.1.3.5. Nonobese Goto-Kakizaki (GK) rats

The GK rat is a nonobese T2D model that develops glucose intolerance at 2 weeks of age and significant hyperglycemia by the age of 4–6 weeks [37]. The rats were generated from repeated

selective inbreeding of normal Wistar rats based on their glucose intolerance indices [37]. After 1 month of hyperglycemia, the rats displayed significantly increased retinal mean circulatory time and reduced retinal segmental blood flow [38]. BRB permeability was increased after 3 months of hyperglycemia [39]. Eight-month-old rats presented with an increased endothelial/pericyte ratio that increased with the duration of hyperglycemia [40]. At 28 weeks, there were increased ocular VEGF concentrations, with significant immunoreactivity in the choroid [41]. This corresponds to the VEGF localization in the eyes of diabetic patients [41]. With 12 months of chronic hyperglycemia, microglia were activated and migrated into the subretinal space between the outer segment and RPE [42], as seen in human proliferative diabetic retinopathy (PDR) with diabetic maculopathy [43]. These retinal microcirculatory changes, however, were not accompanied with retinal vessel diameter or morphological changes. The stable diabetic state of this model allows for the investigation of retinal changes over an extended duration of diabetes.

1.1.3.6. Spontaneously diabetic Torii (SDT) rats

The spontaneously diabetic Torii (SDT) rat is a type 2 diabetic inbred strain of the Sprague-Dawley rat generated by selective breeding of SD rats with polyuria and glucosuria. The rats display glucosuria, hypoinsulinemia, and hyperglycemia without signs of ketonuria. There are prominent sex differences in diabetes development in these rats. Female and male rats develop hyperglycemia by 10 and 20 weeks, respectively, but 100% cumulative incidence of diabetes was only observed in male 40-week-old rats [44]. Merely, 33.3% of female rats developed diabetes by 65 weeks of age [44].

The SDT rat is one of the few rodent models that display advanced lesions and features resembling PDR with prolonged hyperglycemia. Increased cellular apoptosis in the GCL and INL and increased GFAP expression spanning the entire retina [45] were evident at the age of 40 weeks. There was increased retinal leukostasis [46] and retinal thickening [47] by the age of 44 and 52–60 weeks, respectively. Long-standing hyperglycemia in rats older than 60 weeks of age has been shown to result in the distortion of the retina, fluorescein leakage around the optic disc, vessel tortuosity, capillary nonperfusion, neovascularization, and tractional retinal detachment accompanied by proliferative fibrous formations [48] (70 weeks) [44, 49]. Anterior chamber hemorrhage with fibrous proliferation around the iris and posterior synechia formation were also noted in a few cases [44, 49]. A major discrepancy between the rat model and human DR is the development of retinal neovascularization and proliferative DR features in the SDT rat in the absence of retinal ischemia [50]. The presence of microaneurysms and areas of vascular nonperfusion were also rarely reported in this model. Retinal dysfunction was evidenced by delayed OP peak latencies at 32 weeks of age [48] and reduced a-wave, b-wave, and OP amplitudes with increased OP implicit times at the age of 44 weeks [51].

The SDT rat has a relatively long lifespan without requiring exogenous insulin administration. Hence, it may serve as a unique rodent model for PDR, considering that the SDT rate retinae can be exposed to high concentrations of VEGF for a longer period of time. Some researchers have further introduced the *fa* mutation of the leptin receptor gene found in ZF rats into the SDT rat to create a SDT fatty rat that develops diabetes and diabetes-associated complications at a younger age.

1.1.3.7. TetO rat

The NTac:SD-Tg(H1/tetO-RNAi:Insr) (TetO) transgenic rat is a newly reported rat model of T2D exhibiting both hyperglycemia and hyperinsulinemia [52]. The insulin receptor (INSR) gene is reversibly knocked down through the use of tetracycline-inducible small hairpin RNA (shRNA) expression systems [53]. INSR expression is inhibited upon doxycycline administration in drinking water, resulting in insulin resistance. After 4–5 weeks of hyperglycemia, RGC and pericyte loss were observed. The rats displayed an increase in the number of stenotic vessels and veins with abnormal caliber. Blood-retina barrier (BRB) disruption was evidenced by albumin extravasation and reduced zonula occludens-1 expression in the RPE. Increased expression of GFAP and inflammatory markers was also observed in the rat retinae. Functionally, ERG studies revealed significantly decreased scotopic a-waves, b-waves, and c-waves, and an elevated b/a-wave ratio.

The reversible nature of the tetracycline-inducible shRNA system allows for fine modulation of the degree of hyperglycemia to be induced in the rats. This may be useful for the reproduction of clinical situations in an animal model. The rats also project an array of early and late features of human DR within a shorter time span as compared with other rodent models of DR. Given that only one study to date has documented retinopathy changes in the TetO rat, no detailed reports of the timeframe of pathophysiological changes in the retinae are currently available.

1.1.4. Angiogenesis

1.1.4.1. Oxygen-induced Retinopathy (OIR)

In Sprague-Dawley rats, the OIR model predominantly induced vascular changes in the peripheral retina. Two days after pups were transferred to normoxia, there was evident perivascular astrocyte loss [54]. This was followed by significantly increased vascular permeability until P18 [55]. At P18, there was significant reduction in INL, inner plexiform layer (IPL), and total retinal thickness [56] and increased arteriole tortuosity [57]. Increased Müller cell gliosis, as demonstrated by increased GFAP immunoreactivity, was particularly prominent in avascular areas of the retina [56]. Although pericyte apoptosis, a prominent feature of DR, was not observed, pericyte-endothelial interactions were abnormal [54]. This may likewise compromise vascular wall stability. Intravitreal neovascularization was accompanied with decreased blood vessel profiles in the outer and inner plexuses and abnormal vessel tufts extending from the superficial plexus in an extraretinal direction [54, 58]. Functionally, a- and b-wave amplitudes on ERG were reduced in OIR rats [55]. It has been suggested that the degree of vascular hyperpermeability, development of vascular pathologies, and area of avascularity differs, according to the strain of mice used and the degree of VEGF overexpression [55, 59]. Researchers should be meticulous in their selection of rat strains to ensure that desired phenotypes are displayed.

1.2. Nonrodent models

Nonrodent models, including dogs, cats, rabbits, pigs, and zebrafish, have been used for DR studies. Larger animals have eyes that are more similar to human eyes in terms of size and

structure. Larger eyes also allow for the extraction of retinal cells and components of the vitreous for further *in vitro* studies. Nonhuman primates are ultimately most similar to humans anatomically, particularly in regard to the presence of the macula. However, significant costs, ethical challenges, genetic intractability, and feasibility issues regarding the housing and handling of such large animals greatly limit their use. Further studies are required for the establishment of reported features in the following nonrodent models due to the small sample sizes and large intra-species variations in existing reports.

1.2.1. Dog models

Diabetes has been induced in dogs via STZ injections or galactose feeding. Both diabetic and galactose-fed galactosemic dogs display retinal lesions similar to those of human DR, though a long duration of galactose feeding is required for the development of vessel changes associated with PDR. Diabetic dogs displayed capillary BM thickening within 1 year of hyperglycemia, pericyte and smooth muscle cell loss at 4 years, and microaneurysms, acellular capillaries, and IRMAs after 7 years of diabetes [60]. Similar lesions were found in alloxan-induced diabetic dogs after 5 years of diabetes, though such changes were significantly more evident in galactosemic dogs [61]. Galactosemic dogs have been reported to develop features of both NPDR and PDR [62–65], including intravitreal retinal vascular growth and partial detachment of the posterior vitreous after 84 months of 30% galactose feeding [62]. Some have suggested that pericyte destruction and microaneurysm formation are age dependent, with younger animals developing such symptoms at a faster rate than older animals [63].

1.2.2. Cat models

The cat eye has a retinal and choroidal circulation comparable to that of the human eye [66]. The feline lens advantageously does not develop diabetic cataracts, thus allowing for clear visualization of the fundus [67]. The majority of studies of diabetic cats involve induction of diabetes via pancreatectomy. Among the few studies published, there are discrepancies regarding the presence of reported lesions. Within the small sample size, some observed microaneurysms, capillary BM thickening, increased vessel tortuosity, decreased b-waves, capillary nonperfusion, and small intraretinal hemorrhages [68–71], while other cats did not develop microaneurysms or hemorrhage [68]. It is worth noting that the inner half of the diabetic retinae becomes hypoxic prior to the development of apparent capillary nonperfusion. This may be comparable with hypoxia-stimulated VEGF expression in humans prior to the development of DR [67]. The use of cats for DR studies is less feasible due to the slow development of lesions and high costs.

1.2.3. Rabbit models

Although the rabbit globe is significantly larger than that of rodents, rabbits possess a merangiotic retinal circulation, where the inner retina is supplied by choriocapillaris [66]. Humans, rodents, dogs, cats, pigs, and primates have a dual holangiotic circulation, where the inner retina is supplied by central and cilioretinal arteries, and the outer retina is supplied by the

choriocapillaris. Pharmacological, diet-induced hyperglycemia and hypercholesterolemia (lard, sucrose, and cholesterol-added chow [72]), and VEGF injection models (implantation of a human recombinant VEGF pellet into the vitreous [73, 74]) have been used in rabbits for DR studies. However, the presence of DR lesions is highly variable between and within models. Rabbits with STZ-induced diabetes demonstrated varying degrees of retinopathy after 135 days of hyperglycemia. Some showed proliferative retinopathy, while others only demonstrated moderate vasculopathy with hemorrhages and hard or soft exudates [75]. Diet-induced hyperglycemic and hypercholesterolemic rabbits only exhibited an increased number of microaneurysms after 12–24 weeks of special feeding. The development of vascular pathologies and neovascularization in the VEGF implant model was limited by the gradual depletion of VEGF [73]. Neovascularization developed 14 days after the implant was inserted, but there was almost complete regression by 35 days. The addition of basic fibroblast growth factor (bFGF) to the pellet in another study demonstrated a faster induction of retinopathologies [74], but angiogenic responses may vary depending on the strain of rabbits used.

1.2.4. Porcine models

The retinal structure of the pig eye, with its holangiotic retinal circulation and area centralis comparable to the human macula, closely resembles that of the human eye [66]. Few studies have examined morphological changes in the pig retinae. Alloxan-induced pigs developed Müller cell gliosis at 90 days of hyperglycemia [76]. By 20 weeks, pigs developed capillary BM thickening [77], pericyte loss, and capillary collapse with a reduction in the total number of BRB capillaries [78]. STZ-induced pigs showed similar results, with capillary BM thickening and lamellation and rarefaction on electron microscopy at 20-week post-STZ treatment [79]. A proliferative vitreoretinopathy swine model has also been developed, whereby vitreal and retinal detachments were induced surgically prior to the intravitreal injection of RPE cells [80]. However, it is not a suitable angiogenesis model for DR.

1.2.5. Nonhuman primates

1.2.5.1. Monkey models

Monkeys are the most common primate models used for DR studies. Investigations in 39 STZ-induced, pancreatectomy-induced, or spontaneously hyperglycemic T1D monkeys revealed only mild ocular changes after 6–15 years of diabetes [81]. There were no signs of PDR. Only diabetic monkeys that spontaneously developed hypertension or those that were pharmacologically induced to develop hypertension displayed ischemic retinopathy changes, including microaneurysms, capillary dilatation and dropout, focal intraretinal capillary leakage spots, cotton wool spots, and arteriolar lesions. Spontaneously obese type 2 diabetic monkeys developed various retinal lesions and displayed signs of retinal functional loss on ERG, but lesion development was highly variable among individual monkeys [82, 83]. Occurrence of retinopathy was significantly correlated with the presence of hypertension, as seen in the type 1 diabetic monkey models. Angiogenesis monkey models have also been reported, where repeated intravitreal VEGF injections resulted in vessel tortuosity, dilation, leakage, microaneurysm

formation, venous beading, capillary nonperfusion, and preretinal neovascularization [84]. Both VEGF injections and implants resulted in iris neovascularization [73, 85]. Despite their anatomical similarities to human eyes, DR development is highly variable and exceedingly slow, making it an impractical model for DR studies.

1.2.5.2. Marmoset models

An alternate primate model is the marmoset. The marmoset has large eyeballs in respect to its body size with anatomical features closely resembling those of the human eye. The only study to date characterizing the development of retinal DR lesions in these animals reported the development of microaneurysms with increased acellular capillaries, pericyte loss, BM thickening, BRB breakdown, and macular edema in marmosets that were put on a 30% galactose-rich diet for two and a half years [86].

1.2.6. Zebrafish models

Zebrafish (*Danio rerio*) uniquely offers the tractable genetics of rodent models and a retinal structure resembling that of the human eye. They have the distinctive five-layered human retinal structure, though they lack the subretinal plexi found in the inner and outer retina of human retinae [87, 88]. Despite lacking a macula, the density and number of cones in the zebrafish are comparable to that of humans.

Researchers have developed high-glucose diabetic and angiogenic models to mimic DR. Hyperglycemia was induced by immersing zebrafish into glucose-added water [89, 90]. This resulted in reduced IPL and INL thickness [90] and increased diameter of hyaloid retinal vessels [33]. Zebrafish subjected to a hypoxia-induced retinopathy model developed neovascular features after 3 days [91]. One group developed an angiogenesis model using *vhl* (von Hippel-Lindau tumor suppressor gene) mutant fish. By inactivating the *vhl* tumor suppressor gene, hypoxia-inducible factor was upregulated, resulting in overproduction of VEGF. Extensive neovascularization and PDR features were observed in these *vhl* mutant zebrafish, but such a model is not commercially available.

The short lifespan, rapid development, and capability of zebrafish to breed in exceedingly large numbers allow for high-throughput screening [88], genetic screening, and significantly shorter experimental turnover times as compared with that of rodents. Techniques for developing transgenic lines and gene-targeting mutations have also been established. Although the presence of DR-like lesions and exact models ideal for the simulation of DR have not yet been well established, the zebrafish represents a promising short-term model for future DR drug screening.

2. Next-generation DR models and conclusion

The ideal animal model for DR would mimic the complete pathophysiological process of DR in humans, with initial development of NPDR features and gradual progression to PDR with

or without macular edema in the presence of prolonged hyperglycemia. However, the majority of existing animal models only display early or advanced lesions of DR. DR has a complex etiology, with various genetic and environmental factors implicated in its disease susceptibility and progression. Animal models are particularly important for the screening of novel therapeutic interventions. The clinical application of gene therapy for DR has been garnering increasing interest, and this may call for animal models that better reflect the intricacies of DR pathogenesis. Mice and rats are highly genetically tractable, but current transgenic models are based on isolated crosses [92]. Increasing development and use of resources such as The Collaborative Cross [92] may be required to enhance future identification and development of mouse strains with complex traits and epigenetics that are more reflective of the clinical scenario. Only by identifying and manipulating genes that mediate clinically relevant phenotypes can we realize and exploit the full genetic tractability of the mouse to better model DR.

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