Models of Diabetic Retinopathy

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Contributor: Marina Gorbaytuk

Diabetic retinopathy (DR) is an ocular complication of diabetes mellitus (DM), a metabolic disorder characterized by elevation in blood glucose level. The pathogenesis of DR includes vascular, neuronal, and inflammatory components leading to activation of complex cellular molecular signaling. If untreated, the disease can culminate in vision loss that eventually leads to blindness. Animal models mimicking different aspects of DM complications have been developed to study the development and progression of DR. Despite the significant contribution of the developed DR models to discovering the mechanisms of DR and the recent achievements in the research field, the sequence of cellular events in diabetic retinas is still under investigation. Partially, this is due to the complexity of molecular mechanisms, although the lack of availability of models that adequately mimic all the neurovascular pathobiological features observed in patients has also contributed to the delay in determining a precise molecular trigger.

animal models of diabetes | diabetic retina | electrophysiology of diabetic retina | cellular signaling of diabetic retina | tribbles homolog 3 protein

1. Introduction

Diabetic retinopathy (DR) is known to be an eye complication of diabetes mellitus (DM). If untreated, it can threaten the vision of affected individuals. Current clinical trials using in vivo imaging techniques have reported dramatic retinal morphological changes associated with diabetes. A study with 124 human subjects in the early stage of DR reported a decrease in the thickness of the nerve fiber layer (NFL) with no effects to the outer neural layer (ONL) of the retina, measured by spectral domain optical coherence tomography (SD-OCT) [1]. Electroretinographic changes have also confirmed retinal dysfunction in patients with severe ocular diabetic complications [2][3]. Furthermore, changes in retinal hemodynamics have also been reported in patients with early DM [4]. Currently, DR is recognized as a progressive neuro-vascular complication with neuronal dysfunction proceeding to microvascular damage [5]. The early stage of the disease is known as non-proliferative diabetic retinopathy (NPDR); it ranges from mild (microaneurysms) to severe (decrease in the blood flow due to blockade in a larger section of retinal blood vessels). Proliferative diabetic retinopathy (PDR), an advanced stage in which blood vessels grow in the retina, often leads to a reduced field of vision and blindness. While clinical trials concentrate on risk factors, early detection, and evaluations of the progression of DR in vivo, access to human donor eye tissue provides a great opportunity to study early molecular changes in the diabetic retina to further understand pathological markers. Multiple studies with postmortem donor eyes have reported glial cell dysfunction as a primary change in the diabetic retina. Thus, a recent study with postmortem diabetic ocular tissue that employed an immunolabelling

technique to detect carbonic anhydrase (II) and glial fibrillary acidic protein (GFAP) identified the occurrence of Müller cell reactivation in the human diabetic retina [6][7]. The authors discovered that the Müller cells migrated in the pre-retinal membranes and overexpressed GFAP in the diabetic donor eyes.

In addition to structural and morphological alterations, molecular changes occurring in diabetic retinas have also been reported. Thus, studies on post-mortem diabetic eyes have shown the elevation of inflammatory markers; an increase in pro-death caspase-3, Fas, and Bax in the retinal ganglion cells (RGC); and GFAP in the retina [8][9][10]. Several studies on vitreous extracted from patients demonstrated that levels of interleukin-8, monocyte chemotactic protein-1, macrophage-colony stimulating factor, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) are elevated compared with non-diabetic individuals [11][12][13][14]. In addition, extracellular matrix proteins and an elevated expression of genes associated with angiogenesis and apoptosis were identified in fibrovascular membranes extracted from PDR patients during vitrectomy [15]. These analyses have also helped to identify potential therapeutic targets. Thus, it has been confirmed that VEGF plays an important role in the development of aberrant neovascularization in diabetic retinas and that VEGF is a biomarker of microangiopathy in PDR [16]. In addition to VEGF, increases in the number of apoptotic cells measured by a terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, as well as pericyte and endothelial cell loss, were reported in the retinas of patients with diabetic microvascular complications [17]. Although studies with human donor tissues are an excellent asset for improving our understanding of the molecular signaling contributing to DR pathobiology, they cannot provide a complete picture of the mechanism of the development of DR. Moreover, human donor tissues may not be readily available. The use of genetic animal models addresses these limitations, and they provide an excellent approach to developing a comprehensive understanding of the cellular pathways associated with DR. While the choice of the appropriate animal model that mimics all aspects of human DR pathology is challenging, several models can capture key cellular and physiological events of diabetic retinopathy in humans.

2. Rodent Models of Diabetic Retinopathy

There are several spontaneous hyperglycemic rat models, including bio-breeding (BB) rats developing T1D, Wistar Bonn/Kobori (WBN/Kob), Zucker diabetic fatty (ZDF), Otsuka Long-Evans Tokushima fatty (OLETF), and spontaneous diabetic Torii (SDT) rats developing T2D. The BB rats manifest autoimmune DM and DR based on hyperglycemia registered at three months of age and retinal vascular changes at 8–11 months [18][19]. In WBN/Kob male rats, the onset of hyperglycemia occurs at nine months of age [20]. In contrast, ZDF rats develop hyperglycemia earlier, between 5 and 10 weeks of age. These animals are considered a non-insulin-dependent DM model. They are obese and carry a missense mutation known as *(fa/fa)* mutation in the leptin receptor gene (Lepr). Originally, these rats were derived from the Zucker rats, which are an obesity disease model [21][22]. Male OLETF rats develop high BGL starting at five months [23]. In the SDT rat model, detection of glucose in the urine, which is a common sign of glucosuria and kidney damage in patients, was reported at 20 weeks of age in males and at 45 weeks of age in females [24].

Mouse models. $Ins2^{Akita}$, non-obese NOD, Kimba and Akimba mice developing T1D, and db/db mice developing T2D are the most popular genetic models of DM. $Ins2^{Akita}$ mice have a point mutation in *insulin2* (earlier reported locus *Mody4*), which causes abnormal insulin production by the pancreatic cells, leading to pancreatic cell death. The heterozygous $Ins2^{Akita}$ males are progressively hyperglycemic starting at four weeks of age, while females exhibit mild symptoms of DM. They have an average life span of 305 days and are primarily a model of early retinal complications caused by diabetes in humans [25][26]. Another model of T1D is the NOD mouse. These mice mimic human autoimmune insulin-dependent DM and exhibit CD4 and CD8 T cell-mediated autoimmune destruction of the pancreas [27][28]. Interestingly, there is a gender-based variability in the timeline for the development of hyperglycemia in these mice. Thus, 80% of the NOD females develop hyperglycemia at the age of 12 weeks, while males develop hyperglycemia later, at around 20 weeks of age [28]. The recently developed Kimba mice are a transgenic line (tr029VEGF) that mimics both NPDR and mild PDR [29]. This model is used for breeding with the $Ins2^{Akita}$ mice to generate an $Ins2^{Akita}$/$VEGF^{+/-}$genotype and is known to be a new model for the comprehensive study of the mechanism of DR as a complication of T1D [30]. Another model, homozygous for the mutation ($Lepr^{db}$) db/db mice, manifests signs of T2D and develops hyperglycemia at the age of 8–10 weeks (300 mg/dL B6.BKS(D)-*$Lepr^{db}$*/J, stock#000697) and at the age of 6 weeks (300 mg/dL, BKS.Cg-$Dock7^{m}$ +/+ *$Lepr^{db}$*/J, stock# 000642). These mice are widely used because, in addition to hyperglycemia, they model obesity and metabolic disorders [31].

2.1. Pathological Signs in Rodent Models of Diabetic Retinopathy

2.1.1. Neovascularization and Microvascular Changes in Diabetic Rodents

The most critical pathologic findings of PDR are neovascularization, hemorrhage, and fibro-vascular proliferation, leading to retinal traction and detachment and vitreous hemorrhage [32]. Oxygen-induced retinopathy (OIR) in rodents is an accurate and reproducible model of vascular proliferative changes in the retina [33]. Hypoxia-driven vascular proliferative changes seem to be similar to those seen in the retinopathy of prematurity, age-related macular degeneration, and diabetic retinopathy. OIR was developed in canine models for the first time in the early 1950s. In this model, Arnall Patz and colleagues investigated the effects of hyperoxia on retinal vessel development to study proliferative retinopathy [34][35]. To develop this model, one-day-old pups were exposed to hyperoxia for four consecutive days. In the early 1990s, this approach was introduced in rodents by Dr. Smith and her colleagues and has gained increasing popularity. In addition to OIR canines and rodents, aberrant angiogenesis has also been reported in zebrafish, rabbit, and monkey models.

The rodent OIR model is the most common approach to investigating the effect of hypoxia on the retina since it mimics the characteristics of human retinal proliferative changes [33][36][37]. Because retinal vasculature develops in the first two weeks of birth in rodents, researchers can leverage this opportunity to analyze the aberrant vascular development triggered by hypoxia. In this model, hypoxia is induced at postnatal day (P) 7 after the regression of hyaloid vessels to avoid the development of mixed hyaloidopathy. The rodent pups were then exposed to hyperoxia (75% oxygen) for five consecutive days from P7 to P12 and then observed at room air from P13 to P17 [33]. The peak changes of neovascularization are usually observed at P17, and these are resolved by P25. The

C57BL/6 mice or the Sprague Dawley (SD) rats are the common strains employed in this model due to their neovascular susceptibility to hypoxia [36][37][38]. The OIR mice developed irregular blood vessels and a reduction in the retinal inner and deep plexuses at P18, mimicking retinal proliferative events triggered by hypoxia in patients with diabetic complications [39]. Downie and colleagues reported an increase in extraretinal neovascularization and impaired pericyte distribution in the OIR SD rat retinas as early as P18 [40].

Genetically modified Akimba, Akita, and Kimba mice manifest vascular dysfunction. Akimba mice were specifically developed to study the microvascular changes of DR and showed these changes at the early age of eight weeks old [30]. Thus, at eight months of age, these mice developed neovascularization, retinal edema, and detachment that progressed further through 25 weeks of age [30]. In the Kimba mice, abnormal blood vessel development was seen as early as P28, while an increase in vascular permeability and adherent leukocytes was observed at six weeks of age. Additionally, loss of retinal capillaries, neovascularization, an increased avascular area, alteration in the vessel length, and pericyte loss were reported from nine weeks to the advanced age of 24 weeks [29][41]. Vascular dysfunction in $Ins2^{Akita}$ mice presents as an increase in leukocytosis at eight weeks, compromised vascular permeability at 12 weeks, microaneurysms at six months, and neovascularization at nine months of hyperglycemia [26][42].

STZ mice also show microvascular changes earlier in the course of diabetes compared to STZ-induced hyperglycemic rats. For example, vascular permeability detected by imaging the distribution of fluorescein-conjugated dextran is compromised in these animals as early as eight days post-STZ injection [43]. However, a decrease in arteriolar diameter and velocity were reported at four weeks and eight weeks post-STZ injection, respectively [44]. Later in the course of diabetes (six to nine months), the STZ-induced hyperglycemic mice manifested pericyte loss and developed acellular capillaries [45].

In albino Wistar–Kyoto rats, the blood retinal barrier (BRB) disruption occurs as early as two weeks post-STZ injection. Several studies reported early neovascular changes such as adherent leukocytosis and thickening of the basement membrane occurring at 8 and 12 weeks, respectively [8][46][47]. Gong et al. noted that neovascularization in STZ-injected SD rats can be observed at three to four months after induction of hyperglycemia. An increase in VEGFR1 and VEGFR2 expression levels was associated with neovascularization in STZ-induced rats [48]. Similar findings were observed in the Alloxan-induced diabetic rats; leukocytosis and neovascularization were reported at two and nine months after induction of hyperglycemia, respectively. At two months of sustained hyperglycemia, the authors observed pericyte loss, the formation of acellular capillaries, and basement membrane thickening [49][50]. In contrast, several other studies reported that BB rats with autoimmune T1D manifested these retinal changes as early as four months, while this model as well as genetic ZDF and obese OLETF rat models demonstrated BRB breakdown and pericyte loss at six to eight months [18][19][21][23][51][52]. Overall, these studies imply that the observed vascular dysfunction could vary in rat models of DR triggered by different insults. In addition to rats, hyperglycemia induced by a high-fat diet in db/db mice with T2D also leads to an increase in vascular permeability and basement thickness at 13–14 weeks of hyperglycemia [53][54]. Moreover, these mice also demonstrate pericyte loss, blood retinal barrier disruption, and vascular leakage at 12 months of age [55].

Neuronal cell death and gliosis are observed in the diabetic retina of animals with diabetes. Thus, in hyperglycemic rats, GFAP activation has been reported. STZ injection results in an increase in GFAP immunoreactivity in the retina as early as six to seven weeks [56] and as late as 8–16 weeks post-injection [56][57]. Retinal cell loss and functional changes have also been reported as early as two weeks and as late as 24 weeks post-STZ injection. Moreover, an increase in apoptotic cell death in the ONL, INL, and RGC layers resulting in a decrease in the total retinal thickness has been detected between 12 and 16 weeks post-STZ injections in rats [57][58]. In contrast, necrotic RGC death was reported at four weeks post-STZ treatment in rats [58]. These rats also manifested severe loss of photoreceptors at 12 and 24 weeks, [58] while in WBN/ Kob rat retinas, photoreceptor degeneration occurs earlier, at four weeks of age [20]. Our recent study also confirmed RGC function loss and cell death in STZ-induced hyperglycemic mice at 32 weeks post-injection [59] and tree shrews at 16 weeks post-injection [60]. In addition to retinal neurons, RPE degeneration was reported in diabetic retinas. Thus, in four-month-old diabetic BB rats, hyperglycemia induces RPE degeneration through focal necrosis [61]. In hyperglycemic OLETF rat retinas, the decrease in the thickness of the RPE layer along with a reduction in the INL and ONL thicknesses occurs later, at nine months after induction of hyperglycemia [23]. Much later, at 50 weeks post-hyperglycemia induction, retinal detachment and fibrous proliferation occurs in Torii (SDT) rats with spontaneous diabetes [24]. In other model of spontaneous diabetes, ZFD rats, extensive glial activation along with photoreceptor outer segment (POS) degeneration occurs in 32-week-old retinas [62].The latter agrees with multiple studies demonstrating the thinning of the INL and IPL in OIR rat pups at P18 [40][59][63][64]. In addition, the thinning of the inner limiting membrane (ILM) is observed in STZ-induced SD retinas [61].

In STZ-induced diabetic mice, RGC loss occurs between 6 and 12 weeks [65]. RGC death occurs through apoptosis. The number of RGC apoptotic positive cells measured by TUNEL is 25% higher than that in control retinas [66]. These data are similar to the authors' observation of about a 30% RGC death with this model, [59] although another study reported that the RGC density across the retina varies at 20 weeks post-STZ treatment [67]. A few studies with $Ins2^{Akita}$ mice detected early cone photoreceptor cell loss at three months. The authors observed a significant reduction in the IPL and INL thicknesses along with a diminishing number of RGCs at 22 weeks and 36 weeks of hyperglycemia [26][68]. Similarly, the OCT analysis of 16- and 28-week-old diabetic db/db mice retinas showed thinning in the NFL and RGC layer at a rate of 0.104 µm per week, resulting in a reduction of the total retinal thickness by 28 weeks [67][69]. The 28-week-old diabetic db/db mice also showed TUNEL positive photoreceptor cells and reduction in the ONL thickness. STZ-induced hyperglycemia in mice also leads to GFAP overexpression in retinal astrocytes at five weeks post-STZ treatment, while Müller cell gliosis are not seen even after 15 months of DM [45][70]. In contrast, the OIR mice demonstrated a reduction in the total retinal, INL, and IPL thicknesses, as well as distorted photoreceptor OS, neuronal loss, hyperactivity of Müller cells, and microglial activation at P18 [39].

2.1.2. The Detection of Functional Changes of the Neural Retina in Diabetic Rodents

Several studies with diabetic rats have reported ERG findings. First, there is a delay in the implicit time detected at four to seven weeks post-STZ. Second, a decrease in the a-wave of the scotopic ERG amplitude was detected at 10 weeks, while the b-wave amplitude was found to be reduced at 25 weeks after the induction of hyperglycemia

[56][71][72][73]. Similar ERG findings were observed in SDT rats at 44 weeks post-STZ treatment [74][75] and mice and rats with proliferative retinopathy at P18 [39][40][63][64]. In the STZ-treated mice, retinal functional test showed a decrease in the implicit time for OP at 4-6 weeks, reduction in the scotopic ERG a and b-wave amplitudes at six months and diminished photopic ERG negative amplitudes at eight months after the induction of hyperglycemia [59][76][77][78]. Moreover, db/db (Lepr ^{db}), Ins2^{Akita}, and high fat diet-induced diabetic mice manifested similar retinal function changes detected by ERG at 6, 9 and 12 months, respectively [55][42][68][79].

2.2. Cellular Signaling Changes in the Diabetic Rodent Retina

2.2.1. Insulin Signaling in the Diabetic Retina

Basal insulin receptor (IR) signaling has been extensively studied in the STZ-induced diabetic SD rat retina. It has been observed that the phosphorylation of insulin receptor (IR) in hyperglycemic retinas remained unchanged up to eight weeks post-injection, whereas PI3K activity was reduced by 25% compared to the controls. At 12 weeks post-STZ injection, both kinase activity and auto-phosphorylation of the IR were significantly decreased, suggesting that the basal IR activity is diminished in the diabetic retina. It was also demonstrated that Akt1 kinase activity was significantly diminished at eight weeks post-STZ injection, suggesting compromised glucose flux [80]. Kondo and colleagues observed important differences in insulin signaling between STZ-induced hyperglycemic mice and db/db mouse models developing DR. Specifically, IR expression and tyrosine phosphorylation were upregulated the first week post-STZ treatment in mouse retinas, but no changes were observed in 8- to 10-week-old db/db mice. Moreover, IRS-1 expression was unaltered, while IRS-2 expression was increased in both db/db and STZ-induced diabetic mouse retinas. In contrast, a few studies have reported a reduction in IR phosphorylation and an increase in the activity of the protein tyrosine phosphatase-1B (PTP1B) in the rod's inner segments one-week post-STZ injection in mice [81]. An analysis of phosphorylated PTP1B in these mouse retinas point to PTP1B as a promising therapeutic target to delay neurodegeneration in diabetic retinas [82]. Reduced IR kinase activity at 12 weeks of hyperglycemia was also reported in a study with Ins2^{Akita} mice [26]. The potential contribution of excess glucose to local impairment of retinal insulin receptors and AKT activity has been proposed [80]. The authors' recent study confirmed an excess of glucose in diabetic retinas [59]. Moreover, other studies have reported reduced AKT phosphorylation as an early event in diabetic retinas with T1D [80][83] and T2D [84][85].

2.2.2. Unfolded Protein Response (UPR) and Inflammation in the Diabetic Retina

Endoplasmic reticulum (ER) stress is one of the important features of the molecular pathobiology of the diabetic retina. Three independent UPR arms became activated during the ER stress response in diabetic retinas, including PKR-like ER kinase (PERK) and eukaryotic translation initiation factor 2α (eIF2α); inositol-requiring protein 1α (IRE1α)-X-box binding protein 1 (XBP1); and activating transcription factor 6 (ATF6)[86]. Activation of PERK kinase signaling results in phosphorylation (p) of eukaryotic translation initiation factor 2α (eIF2α), leading to global translational arrest and upregulation of activating transcriptional factor 4 (ATF4), C/ERB homologous protein (CHOP), and tribbles homolog 3 (TRIB3) proteins. The triggering of ATF6 is associated with its autophosphorylation, translocation to Golgi, and cleavage leading to active p-ATF6 transcriptional factor. After autophosphorylation, IRE1α, possessing both the RNAse and kinase activities, trims the Xbp1 transcriptional

factor, leading to the formation of activated transcriptional factor, which controls a variety of gene expressions. Cellular stresses such as hypoxia and glucose imbalance can trigger UPR. ER stress markers are upregulated in diabetic rat retinas as early as eight weeks after the onset of diabetes induced by STZ in SD rats [87]. Apoptotic protein caspase 12, CHOP, and phosphorylated c-Jun N-terminal kinase 1 (MAPK) were dramatically upregulated in these retinas. In addition, the elevation of MAPK kinase was detected in RGCs [87]. However, the differences in the expression of the upstream and downstream mediators of PERK signaling, *Grp78* and *Atf4* genes, respectively, were not significant in this study. These findings suggest that AFT4 might not be the only signaling molecule responsible for the increased VEGF level in diabetic retinas [88]. Using immune-histochemical detection, the investigators reported the elevation of HIF-1α, ATF6, XBP1, and CHOP proteins in STZ-induced diabetic rat retinas at two and four months [57]. This elevation was accompanied by a decrease in the autophagy marker LC3B-II levels, indicating a potential reduction in autophagy in the diabetic retinas of mice with four months of hyperglycemia [57]. Pro-apoptotic BAX was detected in hyperglycemic ZFD rat retinas at six weeks of age [89].

Inducing diabetes and DR by STZ injection in mice, Chung et al. and Zhong et al. reported the activation of the ER stress response and pro-inflammatory signaling [90][91]. They found that the diabetic mouse retinas manifest increased expression of GRP78, pPERK, CHOP, VEGF, and peIF2α four weeks after STZ-induced hyperglycemia. Moreover, ATF4 deficiency resulted in altered inflammatory gene expression [91]. In addition to UPR markers, the above-mentioned study reported that MCP-1 and TNF-α expression simultaneously increased in diabetic retinas during the four-week period [90]. Interestingly, this study also highlighted that UPR signaling could be resolved later in diabetic mouse retinas, at six weeks post-STZ injection. In contrast, genetically modified Akita mice demonstrated an increase in p-eIF2α and GRP78 proteins (PERK arm) in addition to elevated IRE-1 and TNFα expression at 12 weeks of age [92][93]. Elevated levels of GRP78, ATF4, and peIF2α were also found in the OIR model at P15 [93][94] and in 15-month-old db/db (Lepr ^{db}) mice [95]. Moreover, this study showed that limiting ATF4 expression in hypoxic retinas significantly reduced the degree of neovascularization in the OIR mouse retinas, [94] and the deficiency in ATF4 could reduce IL-1β in diseased retinas [96].

The authors' recent study with diabetic TRIB3 KO demonstrated that TRIB3 is a master regulator of insulin signaling and glucose metabolism in the retina (**Figure 1**). Thus, the authors revealed that TRIB3 is induced in diabetic retinas, leading to overexpression of HIF1a, GFAP, VEGF, GLUT1, and EGFR proteins. In turn, HIF1a regulates GLUT1 expression and, together with TRIB3, controls the uptake of glucose in the retina. Moreover, TRIB3 mediates the retinal ganglion cell fate decision, while TRIB3 KO results in neuronal survival and improvement of vascular health.

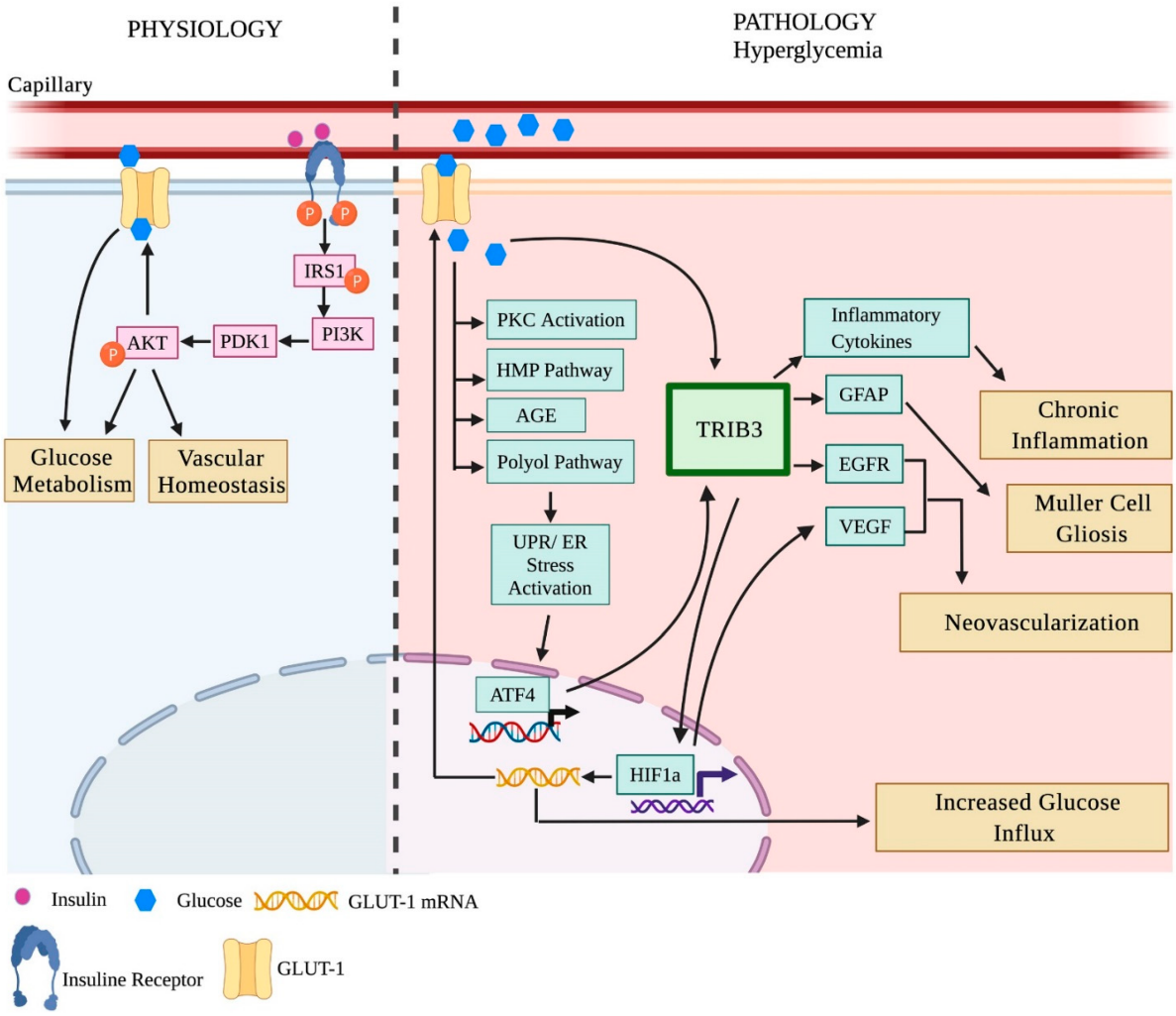

Figure 1. Tribbles homolog 3 (TRIB3) protein controls the development and progression of diabetic retinopathy. The PERK UPR marker TRIB3 is a known psuedokinase that binds and prevents AKT phosphorylation by PDK1. In addition, it controls the expression of HIF1α, EGFR, GFAP, and inflammatory cytokines in cells. In hyperglycemic retinas and retinas of mice with proliferative retinopathy, TRIB3 is significantly upregulated. This results in overexpression of HIF1α, EGFR, GFAP, and inflammatory cytokines (Icam1, Nf-kb1, Rc3h1, Zc3h12a, VEGF, COX2, and AIF1, [59]). In turn, overexpressed HIF1α leads to GLUT1 activation and, together with TRIB3, increases the influx of glucose, which affects the overall glucose metabolism in diabetic retinas. Aberrant glucose flux and hyperglycemia in diabetic retinas are responsible for the activation of PKC, HMP, AGE, and polyol pathways, which eventually leads to chronic UPR activation. TRIB3-mediated pro-inflammatory cytokine expression results in chronic inflammation, GFAP increase leads to the retinal gliosis observed in proliferative retinas, and VEGF elevation triggers neovascularization in the late stages of DR. Image created by Biorender.com, (accessed on 30 May 2021).

Changes in inflammatory gene expression across varied rodent models of DR have also been reported. For example, six-week-old ZFD rat retinas manifest an increase in the levels of TNF-α and NF-kB [89]. Inflammatory proteins such as clusterin, the tissue inhibitor of metalloproteinase (TIMP)-1, β-2 microglobulin, and von Willebrand factor were overexpressed in the SD rat retinas at four weeks and, particularly, at three months post-STZ injection. In addition, the overexpression of fibroblast growth factor-2 (FGF2) was detected in the ONL of diabetic rat retinas at three months post-STZ. It is also worth mentioning that inflammatory changes are strain-dependent in diabetic

rat models. For example, compared to Long-Evans and Brown Norway rats, SD rats show inflammatory changes more similar to those found in human diabetic retinopathy [97]. SD rat pups with OIR were also reported to overexpress inflammatory markers at P16 [36][37].

Overall, the above-mentioned studies emphasize that alterations in cellular molecular signaling often precede retinal pathophysiological events. These findings suggest that dysfunctional insulin signaling, ER stress response, and inflammation are involved in the pathological progression of DR and can be targeted to develop novel cellular therapies for DR (**Table 1**).

Table 1. Cellular signaling, loss of retinal function and integrity in rodent models of diabetic retinopathy.

Molecular Signaling				
	Model	**Changes**	**Duration of Hyperglycemia**	**References**
1.	STZ Rat	Elevated CHOP, Caspase 12, MAPK retinal cytokines	8 weeks	[57][80][87][97]
		Reduced IR kinase activity	8 weeks	
		Elevated retinal cytokines	3 months	
		Reduced IR kinase activity and autophosphorylation and downregulation of IRS-2 & PI3K	3 months	
		Upregulation of HIF-A, ATF-6, XBP1	4 months	
2.	ZFD Rat	Elevated Bax, TNF-α and NF-kappaB	6 weeks	[89]
3.	OIR Rat	Elevated VEGF, PDEG and TNF-α	P16	[36][37]
4.	STZ Mouse	Upregulation of GRP78, pPERK, CHOP, VEGF, pEIF2α, retinal cytokine and TNF-α	4 weeks	[59][81][82][90][91][92][93]
		Elevated IR expression and tyrosine phosphorylation; upregulated IRS-2 and reduced PDK1/ AKT protein levels and phosphorylation	1 week	
		Reduced IR phosphorylation	1 week	
		Upregulation of TRIB3 and inflammatory cytokines (Icam1, Nf-kb1, Rc3h1, Zc3h12a, VEGF, COX2, and AIF1)	4 weeks	
5.	Ins2Akita Mouse	VEGF and TNF-α elevation, increased mRNA expression; protein expression of GRP78 and elevated peIF2α and ATF4 and reduced IR kinase activity	12 weeks	[26][92][93]

Molecular Signaling				
	Model	**Changes**	**Duration of Hyperglycemia**	**References**
6.	Leprdb (db/db) Mouse	Increased IRS-2 expression and reduced PDK1/ AKT protein levels and phosphorylation	10 weeks	[92][95]
		GFAP activation, increased expression of HIF-A, VEGF, GRP78, p-IRE-1, CHOP, Casapase-3 and ATF4	15 months	
Microangiopathy				
	Model	**Changes**	**Duration of Hyperglycemia**	**References**
1.	STZ Rat	Blood retinal barrier disruption	2 weeks	[8][46][48]
		Adherent leukocytes	8 weeks	
		Thickened Basement Membrane (BM)	12 weeks	
		Neovascularization	3–4 months	
2.	Alloxan Rat	Leukocytosis	2 months	[49][50]
		Neovascularization	9 months	
		Pericyte loss, acellular capillaries, and BM thickening	12 months	
3.	BB Rat	Basement membrane thickening	4 months	[18][19][51]
		Blood retinal barrier breakdown	6 months	
		Pericyte loss	8 months	
4.	ZDF Rat	BM thickening, pericyte loss and acellular capillaries	6 months	[21][22]
5.	OLETF Rat	BM thickening, pericyte loss and acellular capillaries	9 months	[23][52]
6.	OIR SD Rat	Increased extra retinal neovascularization and impaired pericyte distribution	P18	[40]
7.	STZ Mouse	Increased vascular permeability	8 days	[44][43][45][59]
		Decreased arteriolar diameter and velocity	8 weeks	
		BM thickening	4–15 months	

Molecular Signaling				
	Model	Changes	Duration of Hyperglycemia	References
		Pericyte loss, acellular capillaries and pericyte ghost	6–9 months	
		Leukocytosis	8 weeks	
		Increased vascular permeability	12 weeks	
8.	Ins2Akita Mouse	Blood vessels in the outer plexiform layer (OPL) and microaneurysms	6 months	[26][42]
		Acellular capillaries, BM thickening and neovascularization.	9 months	
		Abnormal blood vessel development around photoreceptor	P28	
		Increased vascular permeability and adherent leukocytes	6 weeks	
9.	Kimba Mouse	Loss of retinal capillaries, neovascularization, increased avascular area and alteration in the vessel length	9 weeks	[29][41]
		Pericyte loss	24 weeks	
10.	Akimba Mouse	Microaneurysms, neovascularization, blood vessel constriction, beading, vessel edema, capillary dropout, and new vessel formation it the ONL	8 weeks	[30]
11.	OIR Mouse	Irregular blood vessel development and reduced inner retinal plexus and deep plexus	P18	[39]
		Increased vascular permeability and BM thickening	13–14 weeks	
12.	Db/db Mouse	Pericyte loss	18 weeks	[53][54]
		Acellular capillaries	26 weeks	
13.	High-fat diet Mouse	Pericyte loss, blood retinal barrier disruption and vascular leakage	12 months	[55]
Retinal Integrity				
	Model	**Changes**	**Duration of Hyperglycemia**	**References**

Molecular Signaling				
	Model	Changes	Duration of Hyperglycemia	References
1.	STZ Rat	Decreased pre- and post-synaptic photoreceptor ribbon synapses	4 weeks	[56][57][58]
		Increased GFAP reactivity	6–7 weeks	
		Loss of ONL, INL, GCL	12–16 weeks	
		Severe photoreceptor cell loss	24 weeks	
2.	WBN/Kob Rat	Photoreceptor degeneration	4 weeks	[20]
		Severe OS and ONL degeneration	5–14 months	
3.	BB Rat	RPE degeneration	4 months	[61]
4.	ZDF Rat	Decreased OS, damage to amacrine cells and RPE with gliosis	32 weeks	[62]
5.	OLETF Rat	Decreased INL and photoreceptor cells	9 months	[23]
6.	OIR Rat	Reduction in OS, INL, IPL, total retinal thickness, astrocytes and increased muller activity	P18	[40][63]
7.	High galactose Rat	Increased gliosis and reduced INL and OPL	28 months	[61]
8.	STZ Mouse	GFAP hyperactivity	5 weeks	[45][59][65][67][70]
		Reduced ONL, INL thickness	6–14 weeks	
		Total retinal thickness reduced	20 weeks	
		No retinal cell loss and gliosis	8–12 months	
		Reduced RGCs	8 months	
9.	Ins2Akita Mouse	GFAP hyperactivity	8 weeks	[26][68]
		Reduced IPL, INL and cone photoreceptors	3 months	
		Reduced RGCs	22 weeks	
		Decreased presynaptic and post-synaptic photoreceptor ribbons	36 weeks	

Molecular Signaling				
	Model	**Changes**	**Duration of Hyperglycemia**	**References**
10.	db/db Mouse	Reduced NFL and RGCs	16-28 weeks	[67][69]
		Reduced total retinal thickness	28 weeks	
11.	Akimba Mouse	Photoreceptor cell death	28 weeks	[30]
12.	OIR Mouse	Total retinal thickness reduction, distorted photoreceptor OS, neuronal loss, hyperactivity of Müller cells, microglial activation and disrupted INL and IPL	P17-188	[39][59]
Retinal Electrophysiology				
	Model	**Changes**	**Duration of Hyperglycemia**	**References**
1.	STZ Rat	Decrease in OP amplitude	2–7 weeks	[56][74][75]
		Decrease in OP implicit time	7 weeks	
		Decreased a- and b-wave amplitude	10–12 weeks and at 44 weeks	
2.	OIR Rat	Decreased a- and b-wave amplitude	P18	[40][63][64]
3.	STZ Mouse	Reduced OP amplitude and implicit time	4–6 weeks	[59][76][77][78]
		Reduced a- and b-wave amplitude	6 months	
		Reduced PhNR amplitude	8 months	
4.	Ins2Akita Mouse	Decreased OP amplitude, delay in the OP and decreased b-wave	9 months	[26][68]
5.	Db/db Mouse	Delay in the b-wave, delay in the OP implicit time and decreased amplitude of both photopic and scotopic b-wave	16–24 weeks	[69][79]
6.	OIR Mouse	Significant decrease in the amplitude of a- and b-wave	P18	[39]
7.	High-fat diet Mouse	Decreased OP amplitude	12 months	[55]

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