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Thioredoxin Interacting Protein (TXNIP) and Pathogenesis of Diabetic Retinopathy

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Abstract

Chronic hyperglycemia (HG)-associated reactive oxygen/nitrogen species (ROS/RNS) stress and low grade inflammation are considered to play critical roles in the development of diabetic retinopathy (DR). Excess glucose metabolic flux through the aldose reductase/polyol pathway, advanced glycation end product (AGE) formation, elevated hexosamine biosynthesis pathway (HBP), diacyl glycerol/PKC activation, and mitochondrial ROS generation are all implicated in DR. In addition, endoplasmic reticulum stress/unfolded protein response (er-UPR) and deregulation of mitochondrial quality control by autophagy/mitophagy are observed causing cellular bioenergetic deficiency and injury. Recently, a pro-oxidant and pro-apoptotic thioredoxin interacting protein (TXNIP) was shown to be highly up-regulated in DR and by HG in retinal cells in culture. TXNIP binds to thioredoxin (Trx) inhibiting its oxidant scavenging and thiol-reducing capacity. Hence, prolonged overexpression of TXNIP causes ROS/RNS stress, mitochondrial dysfunction, inflammation and premature cell death in DR. Initially, DR was considered as microvascular complications of endothelial dysfunction and pericyte loss characterized by capillary basement membrane thickening, pericyte ghost, blood retinal barrier leakage, acellular capillary and neovascularization. However, it is currently acknowledged that neuro-glia are also affected by HG in diabetes and that neuronal injury, glial activation, innate immunity/sterile inflammation, and ganglion apoptosis occur early in DR. In addition, retinal pigment epithelium (RPE) becomes dysfunctional in DR. Since TXNIP is induced by HG in most cells, its effects are not restricted to a particular cell type in DR. However, depending on the metabolic activity and anti-oxidant capacity, some cells may be affected earlier by TXNIP than others. Identification of TXNIP sensitive cells and elucidating the underlying mechanism(s) will be critical for preventing pre-mature cell death and progression of DR.

Keywords: TXNIP; ROS/RNS stress; Mitochondrial dysfunction; Inflammation; Premature cell death

Introduction: Retinal Architecture

Retina is a part of the central nervous system (CNS) responsible for visual perception and processing [1,2]. The retina is a complex organ and, like the CNS, it is also an immune privilege site [3], which has a blood retinal barrier (BRB) – the inner BRB formed by tight junctions of the endothelium and the outer BRB form by the retinal pigment epithelium (RPE). Retinal capillaries are covered by pericytes at a ratio of 1:1 with endothelial cell [4], which is the highest pericyte coverage in the entire vascular system [5-7]. Retinal neurons are arranged in three distinctive layers – the photoreceptor layer or outer nuclear layer (ONL), which anchors at RPE, the bipolar layer or inner nucler layer (INL), and the ganglion cell layer (GCL) (Figure 1). In addition, inter-neurons are also present in GCL and INL including amicrine and horizontal neurons [8], which are critical for signal integration of photoreceptor, bipolar and ganglion cells. Correspondingly, there are three synaptic plexuses, the nerve (axonal) fiber layer of ganglions (NFL), the inner plexiform layer (IPL) of the bipoplar axons connected to ganglion dendrites; and the outer plexiform layer (OPL) connection between photoreceptors and bipolar neurons. Supporting these neurons are retinal astrocytes in the NFL, radial Muller cells (MC), the major glial type in the retina whose end-feet expand the entire neuro-retina forming inner limiting membrane (ILM) at the vitreous and outer limiting membrane (OLM) at the photoreceptor outer segment (OS). At the ILM and OLM, MC end-feet make gap junctions by connexins and they also prevent macromolecular entry into the neuro-retina from the vitreous and photoreceptor outer segment debris. The other glial cell is the microglia, innate immune cells of the retina, critical for immune surveillance. Microglia are mostly restricted in the NFL and IPL and they may migrate to OPL and ONL upon retinal injury.

Retina is one of the most active organs in the body, which consumes high level of energy (ATP) for its visual processing activity. Glucose and oxygen are the main sources of retinal ATP production; therefore, mitochondrial (MT) oxidative phosphorylation is the key energy source [9]. Mitochondrial ATP synthesis via inner membrane electron transfer chain (ETC) leaks electrons and generate radical oxygen and reactive oxygen species (ROS) as biproduct [10-12]. Furthermore, photo-oxidation can contribute to ROS generation in the retina [13]. Therefore, the retina operates in a highly oxidative environment and requires an efficient anti-oxidant system to prevent MT and cellular stresses.

The blood supply to the retina is from two sources [14]. The outer photoreceptors receive majority of the glucose and nutrient supply from coroidal capillaries via RPE, while the inner retina receives glucose and oxygen supply from three vascular plexuses, namely (i) the superficial plexus at the NFL and GCL at the vitreous surface, (ii) the inner plexus in the IPL and (iii) the deep plexus in the OPL, mostly after injury. The microvascular capillaries in the retina are surrounded by pericytes (PC), astrocytes, Muller cell end-feet, neuronal foot processes, and microglia [15]. Thus, there is a close communication between

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the neuron and blood vessel for continuous supply of nutrients and oxygen to neurons [16]. MC are critical in communicating between retinal cells for homeostasis [17]. Muller cell takes up glucose and nutrient from the blood and supplies metabolites (such as glutamine and lactate) to neurons; at the same time, they are also involved in uptake and detoxification of neutransmitters such as glutamate. MC store glycogen and they can be utilized as energy source under stress. Being an immune privilege organ, the retina thus lacks a lymphatic drainage. MC are critical for removing retinal fluid to the circulation and vitreous and prevent retinal edema [18,19].

Retinal immune privilege is first provided by physical boundaries of iBRB and oBRB and to some degree by Muller cell end-feet connections in the ILM and OLM of the neuro-retina [20-22]. Furthermore, immune suppression is also an active process by secreting soluble anti-inflammatory molecules and neutrotrophic factors such as pigment epithelium derived factor (PEDF), vascular endothelial growth factor (VEGF)-A, brain-derived nerve growth factor (BDNF), glia-derived nerve growth factor (GDNF), interleukin (IL)-10, transforming growth factor (TGF)-β, arginase, VIP (vasoactive intestinal polypeptide), pituitary adenyl cyclase activating peptide (PACAP), and others [23-27]. Thus, diabetes and the associated chronic hyperglycemia may compromise any one of the retinal features leading to cell injury and death, which may ultimately lead to progression of DR.

Diabetic Retinopathy

Most retinal cells are fully or terminally differentiated cells; therefore, a steady supply of nutrient, cell growth and cell cycle control are important for long-term survival [28-31]. Therefore, any metabolic disturbance and physical injury to the retina will be detrimental. As mentioned above, the retina being an immune privilege site has certain disadvantage in inflammation resolution after injury or infection, although the organ may be initially protected from accidental systemic immune attacks. Activated microglia, astrocyte and Muller cell secrete various cytokines and chemokines that may cause neuronal injury and death in an unresolved inflammation, impaired wound healing and fibrosis [32-34]. With such anatomical and physiological features, the retina is one of the frequent targets of microvascular complications of diabetes. Excess glucose metabolic flux into the retina can cause an initial increase in MT aerobic respiration, ATP production and ROS generation causing ER-stress, protein misfolding and MT dysregulation [35-40]. An injury to blood vessel or neuron leads to glial activation, including Muller cell and microglia, and induces altered neurotrophic and pro-inflammatory gene expression. Glial activation may be beneficial in milder and short-term injuries but are detrimental in chronic diseases such as DR [41,42].

Some of the known early hallmarks of DR include aberrant extracellular matrix (ECM) expression and capillary basement membrane thickening [43-46]. This structural change could disturb the cell-cell communication between EC and PC in the blood vessel, which share a common basal lamina. In addition, EC cell-cell adhesion molecules (e.g., VE-cadherin, claudin, zona occludens, beta-catenin) are disturbed leading to blood vessel leakage [47-49]. Similarly, the RPE barrier may be compromised via alterations in its basement membrane components and cell-cell junction molecules [50,51]. In addition, Muller cell and pericyte interaction at neurovascular junctions may also be compromised and infiltrating plasma components can injure neuronal synapses and activate microglia, which are phagocytes [52,53]. Furthermore, Muller cell activation is frequently observed in response to neuronal injury by enhanced radial glial fibrial acidic protein (GFAP) expression in the neuro-retina. Normally, GFAP expression is restricted to the astrocyte or Muller cell end-feet in the ILM or NFL. Muller glia dysfunction and end-feet swelling will disturb retinal homeostasis since aquaporin 4 and potassium channel are expressed in these cells, which are critical for removal of water from the retina [18,54].

Diabetic retinopathy (DR) is now considered as a neurovascular disease that affects most cells in the retina including capillary endothelium, pericyte, glia, RPE and neurons. Most diabetic patients will develop some form of non-proliferative or proliferative complications of DR during the course of diabetes with 10 to 15 years depending on genetic background and diabetes management. DR has long been considered late pathologies of the microvasculature leading to blood vessel leakage and vascular cell death especially of retinal PC and endothelial cells (EC). Indeed, several studies have shown that vessel pathology is seen at ~6 months in experimental diabetes [55-57]. Conversely, retinal EC inflammation, Muller cell reactivity (gliosis) and early pericyte and ganglion cell death are observed as early as 4-8 weeks in diabetes rodents [38,45] (Figure 1). Nonetheless, it is still unclear whether the vascular damage or the neuronal injury occurs first, and, if any, whether they are related to each other or parallel events. Nonetheless, we can assume that either vascular damage or neuronal injury will have a profound effect on each other since they are closely link to retinal metabolism and function. At present,

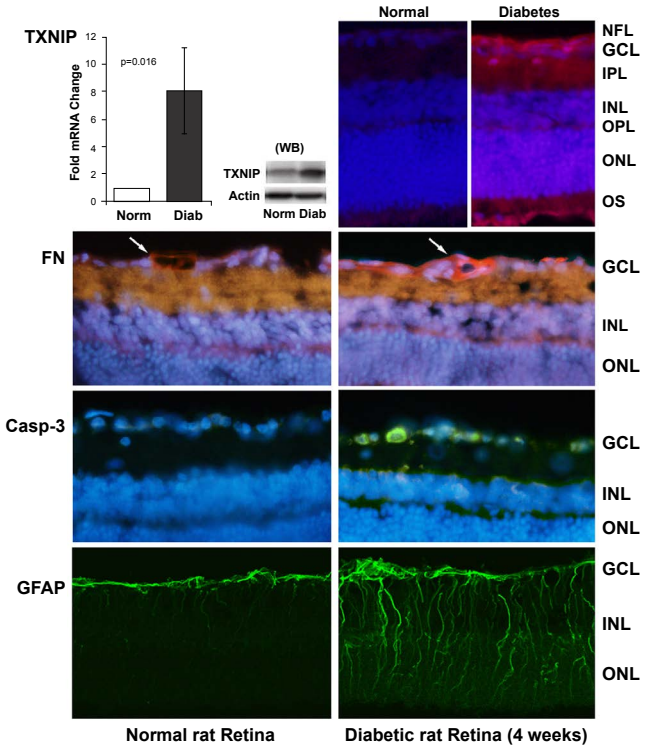

Figure 1: TXNIP overexpression and retinal injury in early diabetic retinopathy. (i) TXNIP is significantly induced in the STZ-induced diabetic rat retina at 4 weeks. **(ii)** TXNIP overexpression is associated with fibronectin (FN) expression and capillary basement membrane (BM) thickening, and **(iii)** neuronal apoptosis both at the GCL and INL as revealed by caspase-3 (Casp-3) expression (adapted from Perrone et al., 2010) [45]. **(iv)** Retinal injury causes Muller glial activation (gliosis), which is represented by radial glial acidic protein (GFAP) expression expanding the entire neuro-retina [38]. These early molecular abnormalities in the diabetic retina are ameliorated by TXNIP knock down via intravitreal siRNA delivery [38,45].

molecular and cellular mechanisms for the initiation and progression of DR are not yet fully understood. Currently, it is recognized that the molecular abnormalities of DR begin early before clinically detectable pathologies appear in the retina. The pathologies once set in motion are not reversed even after glucose normalization [55-57]. Therefore, the detection of early molecular abnormalities and cellular dysfunction that ultimately lead to later pathologies of DR is of utmost importance in developing therapeutic strategies to prevent/slow down blinding ocular complications of diabetes.

Coming to the main topic of this article, the role of the pro-oxidant and pro-apoptotic thioredoxin interacting protein (TXNIP) in DR is currently limited to few publications, especially those originating from our laboratory [38,45,58,59]. TXNIP is highly induced in the diabetic retina and plays a critical role in DR pathogenesis. In this review article, I present a general view of the role of TXNIP in diabetes and metabolic deregulation and put forth an argument for a potential role of TXNIP in the pathogenesis of DR and that TXNIP blockade may ameliorate ocular complications of diabetes.

TXNIP overexpression in diabetes

TXNIP was first identified as a 1,25-dihydroxyvitamin D-3 inducible gene in HL-60 cells, therefore, named vitamin D3 up-regulated protein 1 (VDUP1) [60]. The VDUP1 cDNA was cloned and hybrized to a 2.9 kB mRNA and *in vitro* expression produced in a 46-kDa protein. Using yeast two-hybrid, VDUP1 was determined as a thioredoxin (Trx)-binding protein, and designated as thioredoxin-binding protein-2 (TBP-2) [61]. TBP-2 binds to the thiol active sites of reduced Trx but not to oxidized Trx (Figure 2). Furthermore, VDUP1/TBP-2 expression was downregulated in cancer cells and vitamin D3 induction of its expression lead to cell cycle inhibition at G0/G1 and retardation of cell growth; hence it was considered as a tumor suppressor protein [62,63]. In addition, VDUP1/TBP-2 was found to be silenced in human T-cell leukemia virus type-I (HTLV-I)-infected T cells via TXNIP promoter CpG DNA methylation and that 5-aza-2'-deoxycytidine treatment followed by histone deacetylase inhibitors cause re-expression of TBP-2 [64]. TXNIP mutation was first considered to be associated to familial combined dyslipidemia in a mutant mouse strain, HcB-19/Dem (HcB-19) though the disease was found, later on, to be associated with upstream stimulating factor (USF) 1 in the same locus as TXNIP [65-67]. Recently, the term TXNIP is used most frequently than VDUP1 or TBP-2 and hereafter I refer this protein as TXNIP.

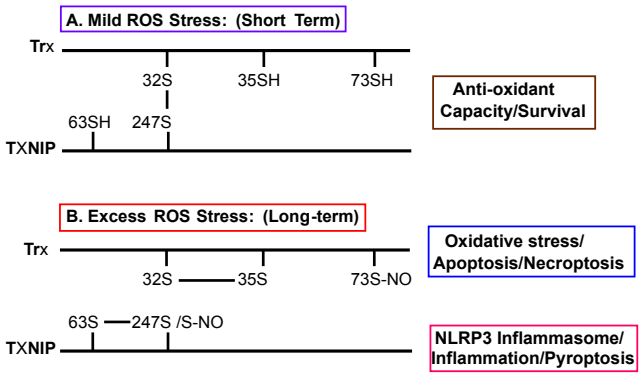

Figure 2: TXNIP binds to Trx and inhibits its anti-oxidant and thiol reducing function. The Trx-TrxR-NADPH axis is critical for cellular redox balance. However, under TXNIP overexpression in diabetes, TXNIP binds to Trx via disulfide bond formation between Trx (Cys 32) and TXNIP (Cys 247) and inactivates Trx activity [177-178]. **(A)** Initially, a mild ROS stress generated by TXNIP-Trx interaction may induce second phage anti-oxidant genes and chaperones such as superoxide dismutases SOD1 and SOD2, catalase and heme oxygenase (HO-1) and heat shock proteins (e.g., hsp70, hsp60, hsp40, and hsp27). **(B)** However, prolonged TXNIP overexpression and excessive ROS stress cause Trx thiol oxidation and cellular ROS/RNS stress leading to programmed cell apoptosis or necroptosis. In addition, release of TXNIP from oxidized Trx1 initiates NLRP3 inflammasome assembly (containing ASC), caspase-1 activation and processing of pro-IL-1β to mature IL-1β. Currently, whether TXNIP is or is not involved in inflammasome assembly is an area of intense research and whether cysteine residues in TXNIP form disulfide bond or *S*-nitrosylation under ROS/RNS stress is yet to be understood.

Using microarray studies, we and others found that TXNIP is one of the highest inducible genes by high glucose (HG) and diabetes in various tissues [68-71]. On the other hand, Trx and Trx reductase expressions were not significantly changed [70]. Trx reduces ROS through its redox active cysteine residues Cys-32 and Cys-35, which are further reduced by TrxR and NADPH [61]. By trapping Trx, TXNIP causes cellular oxidative stress and apoptosis in different cell types in diabetes and under HG, especially of pancreatic beta cells, which is critical for insulin production. Hyperglycemia stimulates TXNIP while insulin and insulin-like growth factor (IGF)-1 suppresses TXNIP expression. Therefore, TXNIP overexpression in pancreatic beta cells in diabetes has profound effect in diabetes initiation and its complications. Shalev's laboratory extensively studied cell death mechanisms of pancreatic beta cells by TXNIP expression in diabetes and under HG in culture [72-74]. These studies provided evidences that, under hyperglycemia and oxidative stress, TXNIP is migrated from the nucleus to mitochondria where TXNIP binding to Trx2 activates apoptosis signaling kinase 2 (ASK2) and caspase-3 dependent cell death of pancreatic beta cells. In mice lacking TXNIP, beta cell mass is increased and protects beta cell death from streptozotocin [73,75].

The physiological role of TXNIP has recently been described as a nutrient sensing and signaling mechanism in the hypothalamus and in peripheral tissues [76-78]. Therefore, it is not surprising that TXNIP is up-regulated in most tissues in diabetes including the retina and plays a critical role in oxidative stress, inflammation, and apoptosis leading to disease progression [38,45,58,59,79]. Furthermore, TXNIP is also a stress response gene and its expression is increased in other retinal diseases including NMDA and amyloid excitotoxicity [80-83]. Therefore, increasing evidence is being presented for a critical role of TXNIP in diabetes and progression of its various vascular and microvascular complications.

TXNIP expression and regulation

TXNIP is an evolutionarily conserved gene expressed from Drosophila to rodents and humans and it is involved in metabolism and development [84]. As mentioned before, TXNIP expression is highly induced by HG, vitamin D3 and glucocorticoid dexamethasone [85,86]. The promoter of TXNIP has several transcription factor binding sites including E-box (also known as carbohydrate response element ChRE), Foxo element [87-89]; and anti-oxidant response element (ARE) [90]. The transcription factors that are involved in TXNIP transcription by HG and cellular stress are ChRE binding protein (ChREBP), MondoA, Mlx, Foxo, NF-Y, HSF-1, and Nrf2. Transcription factors targeting E-box up-regulated TXNIP expression while Foxo1 has been shown to inhibit TXNIP expression in human liver cell line [87]. Conversely, Foxo1 was also shown to increase TXNIP expression in human aortic endothelial cells (HAECs) [89]. On the other hand, Nrf2 binding to TXNIP promoter prevents MondoA binding and reduces the basal and diabetes-induced TXNIP expression in rat cardiomyoblasts [90].

TXNIP promoter exists as an open or poised configuration [45] and histone deacetylase inhibitors activate TXNIP transcription via histone acetylation within minutes [45].

TXNIP induction by HG requires its intracellular metabolism since mannitol or l-glucose at osmolar equivalents of HG (d-glucose) is ineffective in TXNIP induction. Our laboratory has shown that HG effect on TXNIP expression is to some extent mediated by glucose's metabolic flux through the hexosamine biosynthesis pathway (HBP), which modulates protein Ser/Thr-O-GlcNAcylation and gene transcription, especially by targeting transcription factors and protein kinases [38,45,59,70]. Glucose metabolites such as glucose-6-phosphate and glucosamine-6-phosphate, the first metabolite in the HBP, and metabolizable sugars such as 2-deoxyglucose and 3-O-methylglucose can activate TXNIP [29,45,76,91,92]. In addition, adenosine derivatives induce TXNIP expression [93]. TXNIP expression is also activated by 5-aminoimidazole-4-carboxamide ribofuranoside (AICAR), an inducer of AMP kinase; however, AICAR induction of TXNIP is independent of AMP kinase activity and calcium [93]. AICAR effect on TXNIP is mediated by MondoA and requires adenosine uptake and metabolism to adenine nucleotides [93]. In addition, glucocorticoids induce TXNIP up-regulation in pancreatic beta cells and osteoblasts in patients with endogenous Cushing's syndrome [86,94].

On the other hand, agents (such as forskolin and exendin-4) that activate cyclic AMP and PKA suppress TXNIP expression by enhanced degradation as proteasomal inhibitor MG132 prevents forskolin effects on TXNIP protein degradation [95]. The effect of exendin-4 and cAMP appears to involve Epac (exchange protein activated by cAMP) and not by PKA [95]. Inhibition of L-type voltage-gated calcium channel by verapamil also inhibits TXNIP up-regulation in pancreatic beta cells and diabetic cardiomyopathy [96] and in NMDA-induced retinal excitotocity [81].

TXNIP function

The physiological role of TXNIP is not fully understood yet. However, the fact that TXNIP is highly induced by HG and its metabolic products and that TXNIP is inhibited by insulin suggest its role in glucose sensing and metabolism, especially, in peripheral tissues where glucose transporter 1 (glut1) is predominant and are largely insulin independent [97]. In these tissues, high levels of circulating blood glucose could result in excess intracellular uptake in endothelial cells that line the blood vessel. TXNIP may function initially to limit excessive glucose uptake under these conditions by trapping glut1 in the cytosol. Once insulin is released into the circulation by pancreatic beta cells and lowers circulating blood glucose level via uptake by insulin sensitive tissues (glut4 cells) such as muscle, liver, and fat where excess glucose is stored as glycogen, then the level of TXNIP expression will return to the basal level. In the absence of insulin (type 1 diabetes) or insulin resistance (obesity and type 2 diabetes), hyperglycemia persists and TXNIP remains up-regulated causing abnormal glucose metabolic fluxes, defects in MT ETC, energy production, oxidative/nitrosative stress and apoptosis. Here, it is likely that TXNIP by binding to Trx reduces its anti-oxidant and thiol reducing function and causes altered protein cysteine nitrosylation (SNO) [38,58]. Trx is critical for denitrosylation of thiol active cysteines in proteins and enzymes. Trx1 and TrxR1 are expressed in the cytosol and nucleus while Trx2 and TrxR2 are expressed in the mitochondria. TXNIP is expressed in the cytosol, nucleus, MT and plasma membrane; therefore, TXNIP can target multiple organelles and cellular processes under chronic hyperglycemia and diabetes (Figure 3).

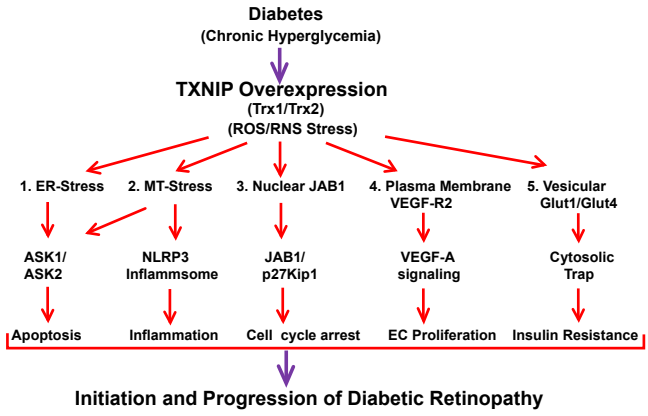

Figure 3: TXNIP overexpression and deregulation of cellular processes. TXNIP is known to express and/or translocate to various organelles under stress including the cytosol (and ER), mitochondria (MT), nucleus and plasma membrane. Similarly, Trx1 is present in the cytosol and nucleus while Trx2 is the mitochondrial isoform. Inhibition of Trx1 and Trx2 by TXNIP releases ASK1 (**1**) from ER and ASK2 from MT, respectively, and induces apoptosis. **(2)** In addition, damaged/depolarized mitochondrial release ROS, which is critical for NLRP3 inflammasome assembly and inflammation. **(3)** In the nucleus, TXNIP releases JAB1 from p27kip1 binding via trapping Trx1 and prevents p27kip1 degradation. P27kip1 is a cyclin-dependent protein kinase CDK4/6 inhibitor, and mediates G0/G1 cell cycle arrest. **(4)** Furthermore, translocation of TXNIP to the plasma membrane causes VEGF-R2 stabilization, VEGF-A signaling, and EC proliferation. This process may enhance retinal capillary neovascularization. **(5)** Lastly, TXNIP causes insulin resistance by trapping glucose transporters glut 1 and glut 4 (or neuronal glut 3) containing vesicles in the cytosol. Therefore, prolonged TXNIP up-regulation in diabetic retinopathy will have deleterious effects in organelle function and cell viability.

Cytosol: In the cytosol, TXNIP can interact with Trx1 and cause protein misfolding in the cytosol and ER lumen inducing ER-stress and unfolded protein responses [38,70,98,99]. Initially, er-UPR is cytoprotective by enhancing protein folding but irreversible ER-stress enhances TXNIP mRNA stability, Nod-like receptor NLRP3 inflammasome activation, and ASK1-mediated apoptosis in pancreatic beta cells [98,99]. One of the potential targets of TXNIP in the ER lumen is the *S*-nitrosylation of protein disulfide isomerase (PDI), which is critical for protein oxidation-reduction and disulfide bond formation in the ER [100,101]. In addition, TXNIP can mediate cytosolic protein oxidation and apoptosis such *S*-nitrosylation of glyceraldehydes 3 phosphate dehydrogenase (GAPDH) and matrix metalloproteinase (MMP) 9 [100,101]. Furthermore, TXNIP reduces mTOR activity by increasing Redd1 level, which could result in coordinated increases in autophagy, nutrient recycling, and protein synthesis [102]. Redd1, which is induced by hypoxia and DNA damage, is a negative regulator of mTOR, therefore, blocks protein synthesis and autophagy.

Mitochondria: In mitochondria, TXNIP interacts with Trx2 causing MT oxidative stress, bioenergetics imbalance, autophagy/mitophagy, and apoptosis [38,74,103,104]. Mitophagy is a mechanism for removing damaged mitochondria via a double membrane autophagolysosome and degradation of the contents for recycling [105]. In this regard, TXNIP may participate in autophagy and mitophagy in several ways (Figure 4). First, TXNIP by trapping Trx1 releases phosphatase and tensin homolog (PTEN) and hydrolyzes membrane phosphatidylinositol (PtdIns)-3-phosphates, which activates PI3-kinase/Akt signaling [106,107]. PTEN activates parkinson-related proteins, PINK1 and PARKIN, and causes mitofusin (Mfn) 2 ubiquitination and degradation. Mfn2 is important for MT fusion [108,109]. In addition, *S*-nitrosylation of dynamin-related protein

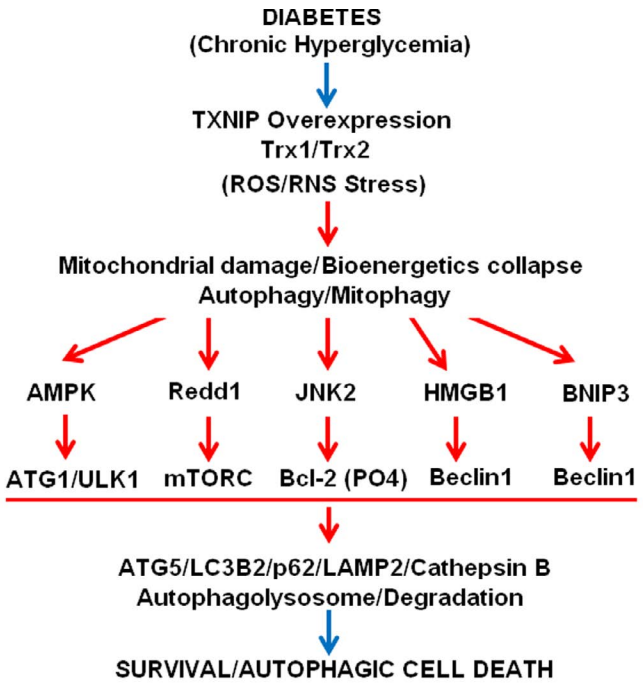

Figure 4: A potential role for TXNIP in the initiation of autophagy/mitophagy. Macroautophagy or autophagy is a catabolic process of degradation and removal of cytosolic misfolded/aggregated proteins or damaged organelles such as mitochondria (mitophagy, autophagy of mitochondria) by a double membrane [105]. **(i)** The process of autophagy/mitophagy may be initiated under starvation by AMP kinase and ATG1/ULK1 or **(ii)** by Redd1 interaction with 14-3-3 protein to inhibit the mTORC1 pathway, which blocks autophagy. **(iii)** In addition, JNK2 kinase activation by cellular stresses mediates Bcl2 phosphorylation and releases beclin1 (or ATG6) to initiate autophagy/mitophagy. **(iv)** Furthermore, oxidation (thiol *S*-nitrosylation) of specific cysteine residues of nuclear HMGB1 causes its cytosolic translocation and releases beclin1 from bcl2. **(v)** Similarly, under hypoxia (and chronic hyperglycemia), HIF-1α is stabilized and induces expression of its downstream target BNIP3 (bcl2 interacting protein). BNIP3 then interacts with the beclin1/bcl2 complex releasing beclin1. **(vi)** Subsequently, autophagy initiation involves ATG5, which modifies and activates microtubule light chain 3B (LC3B) I via lipidation to generate active LC3BII, which initiates double membrane formation. Then, p62 recognizes damaged and ubiquitin-flagged cargoes thereby linking them to LC3BII double membrane to autophagosome. LAMP2, a lysosomal membrane protein, mediates fusion of autophagosome and lysosome (autophagolysosome). Lysosomal enzymes, such as cathepsin B and D, degrade the cargo within autophagolysosomes and releases metabolites for reuse. **(vii)** Mitophagy is critical for mitochondrial homeostasis and cellular survival, especially for differentiated cells such as the RPE and neurons [105,179]. Nonetheless, excess induction of autophagy/mitophagy induces programmed autophagic cell death involving caspase-3 or independently due to cellular energy collapse.

(Drp) 1, a GTPase, leads to MT localization where Drp1-SNO interacts with fission protein 1 (fis1) leading to MT fragmentation. MT fission is a critical step for mitophagy. At the same time, TXNIP also can cause *S*-nitrosylation of nuclear high mobility group protein (HMGB) 1, its nuclear export and cytosolic localization [110,111]. HMGB1 then binds to bcl2 and releases beclin 1 (autophagy-related gene ATG6), which initiates the process of mitophagy. Other mechanisms of autophagy initiation under oxidative stress may include JNK-2 activation and bcl-2 phosphorylation as well as HIF-1α-mediated BNIP3 expression [105].

Mitochondrion being a symbiotic evolutionary bacterium when damaged produces various danger signaling molecules (DAMPS, damage-associated molecular patterns) such as ROS, ATP, mtDNA and formylated MT peptides, which are recognized by intracellular and extracellular pattern recognition receptors (PPRs) including the NLRP3 inflammasome. TXNIP has recently been implicated in pro-inflammatory interleukin-1β (pro-IL-1β) expression, caspase-1 (NLRP3 inflammasome) dependent processing and secretion [38,112,113]. IL-1β is a pro-inflammatory cytokine that orchestrates innate immune responses and sterile inflammation in chronic metabolic diseases. As mentioned above, HMGB1 in addition to its role in autophagy/mitophagy is secreted actively by injured and dying cells as an alarmin (danger signal) and binds to toll like receptor (TLR4) and AGE receptor (RAGE) inducing pro-inflammatory cytokine gene expression. HMGB1 cytokine activity is regulated by oxidation/reduction state of conserved cysteine residues (C23, C45, and C106) [114,115].

Nucleus: TXNIP is also localized in the nucleus. However, TXNIP lacks a nuclear localization signal. It has been shown that TXNIP interaction with the importin system is needed for its nuclear translocation [63]. Compared to the cytosol, the nucleus has a robust reducing environment and TXNIP has been shown to involve in the regulation of Trx1-dependent redox regulation of transcription factors [116]. In general, cytosolic protein *S*-nitrosylation leads to transcription factor release from its binding partners and translocation to the nucleus. On the other hand, oxidation of transcription factors in the nuclear leads to their inactivation and cytosolic export [117,118]. An example is the case of second phase anti-oxidant transcription factor Nrf2. Under normal physiology, Nrf2 is sequestered by Keap1; however under oxidative stress, Keap1 undergoes *S*-nitrosylation of its cysteine residues and releases Nrf2 leading to nuclear translocation and anti-oxidant gene induction [119,120]. Another potential role of TXNIP in the nucleus is the accumulation of cell cycle inhibitor, p27 kip1 and G0/G1 cell cycle arrest. JAB1 (Jun activation domain-binding protein 1) controls cell cycle progression, proliferation and apoptosis, and involves in genomic instability and DNA repair [121,122]. p27Kip1 is a cyclin-dependent kinase inhibitor and mediates cell-cycle arrest at G0 [123]. Recent studies have shown that JAB1 directly binds to p27kip1 and induces nuclear export and subsequent degradation [122]. Trx negatively regulates two important JAB1-controlled pathways, activation of AP-1 transcription and degradation of p27Kip1, probably through a direct interaction between Trx and C-terminal of JAB1 [124]. Therefore, TXNIP up-regulation in the nucleus may cause Trx1 entrapment and p27 Kip1 accumulation and cell cycle arrest. This nuclear function of TXNIP may be an attempt to maintain cell growth and cycle control under excess nutrient availability, critically important for terminally differentiated cells to resist cell cycle progression and apoptosis. Further studies are warranted on the role of TXNIP in cell cycle control in DR.

Plasma membrane: TXNIP is also considered as a homologue of α-arrestin, which is involved in protein scaffolding, receptor endocytosis and trafficking [125-127]. The arrestin function of TXNIP is considered to be independent of Trx binding as mutation of cysteine S247 to serine, which is critical for Trx binding, preserves the TXNIP arrestin function and inhibits glucose uptake in adipocytes [127]. In addition, TXNIP has also been shown to translocate to the plasma membrane from the nucleus after treatment with oxidative stress inducers [128-130] and interacts with VEGF receptor 2 to enhance human umbilical vein endothelial cells (HUVEC) survival [129]. In this case, TXNIP is trapped in the nucleus by PARP1. A reduction in ADP ribosylation in PARP1 releases TXNIP and migrates to the plasma

membrane where TXNIP is essential for VEGFR2 internalization in Rab5 positive endosome, which is required for endothelial cell growth and angiogenesis [129].

TXNIP and retinal cell injury in DR

The above description of TXNIP regulation and localization within cellular compartments depict the many facets of TXNIP function in cellular processes in physiology and pathology. TXNIP senses cellular metabolic stress and signals danger; therefore, TXNIP is tightly control by hyperglycemia and cellular stressors. Although TXNIP is up-regulated in most retinal cells, TXNIP may function in different ways including regulation of plasma membrane receptor signaling, glucose uptake, nuclear gene expression and cell cycle control, cytosolic ER-stress and MT-stress responses, and TXNIP is critically involved in bioenergetics, inflammation and apoptosis [38,58,59,112]. Nonetheless, the role of TXNIP in DR is still in its infancy and our laboratory has been at the forefront of this exploration so far [38,45,58,59,].

TXNIP and endothelial dysfunction: TXNIP is highly up-regulated in the retina of diabetic rats *in vivo* and in retinal capillary EC by HG *in vitro* and TXNIP induces pro-inflammatory gene expression for ICAM1, Cox-2, VEGF-A, RAGE, and others [45,59]. TXNIP is also induced by disturbed flow (shear stress) in EC and activates pro-inflammatory ICAM1 (intercellular adhesion molecule 1) and VCAM1 (vascular cell adhesion molecule 1) expression and leukocyte adhesion in atherosclerosis [131,132]. Therefore, we can postulate that, in DR, initially as hyperglycemia prevails, glucose uptake increases by retinal cells including EC and neurons and there will be a high demand for MT glucose and oxygen consumption by oxidative phosphorylation and ATP synthesis. This process will be accompanied by increases in iNOS and Cox-2 expression by endothelial and glial cells, producing NO and prostaglandin E2 (PGE2), respectively [133,134]. These diffusible molecules act as arteriolar vasodialators via smooth muscle cell and capillary pericyte relaxation, which will increase blood flow, resulting in increased erythrocyte and oxygen delivery to the retina. Nonetheless, such events will result in erythrocyte stacking, increase in plasma protein viscosity, and reduce blood flow both in capillaries and post-capillary venules causing endothelial shear stress [135]. A combination of the disturbed flow, plasma viscosity, and hyperglycemia may mediate endothelial TXNIP up-regulation, inflammation and leukostasis in DR. The resultant adhesion of neutrophil and monocyte in the endothelium will cause local increases in cytokine and growth factor production, oxidative stress, endothelial inflammation, altered cell-cell and cell-matrix adhesion molecules and BRB leakage as well as glucose-oxygen deprivation and neuronal injury [47,136]. These changes may be normalized to a certain degree by insulin treatment via glucose control and TXNIP suppression, but not all, depending on the severity and diabetes duration [55-57,79,137-139].

We observed that TXNIP expression is highly induced by HG in retinal EC in culture and reduces its level rapidly (within 30 minutes) when glucose is normalized (Figure 5). Similarly, insulin-like growth factor 1 (IGF-1) reduces TXNIP expression under HG environment and the effect last for 4-6 hr. Thus, night time glucose control and regulation of TXNIP expression both in type 1 and 2 diabetes may be difficult to achieve. Prolonged or frequent intermittent hyperglycemia can cause TXNIP overexpression, ER-stress, insulin resistance and inflammation. Achieving normal glucose level in diabetics is highly unlikely and tight glucose control increases the risk of hypoglycemic episodes, which is again deleterious for cardiovascular and neuronal function. Understanding the insulin signaling pathway(s) that suppress TXNIP expression may lead to TXNIP inhibition and slow down the progression of diabetic complications including ocular diseases. So far, the critical role of TXNIP in endothelial inflammation, dysfunction, and pathogenesis of DR is yet to be fully investigated [38,45,58,59].

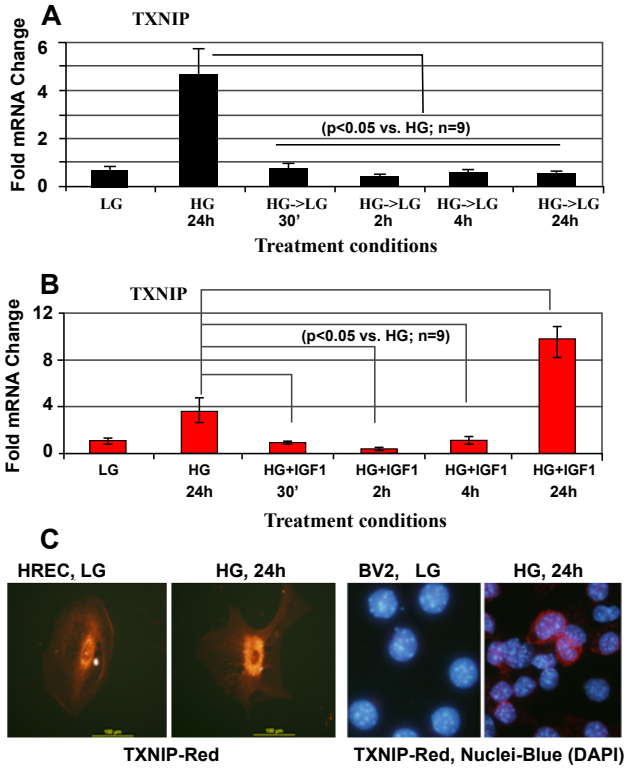

Figure 5: HG induces and IGF-1 blocks TXNIP expression in retinal endothelial cells in culture. (A) A rat retinal endothelial cell line (TR-iBRB2) was maintained under low glucose (LG, 5.5 mM) or high glucose (HG, 25 mM) for 24 in low serum (0.2%) in DMEM media [45,59]. Then, the HG medium was changed to LG and further maintained for different time periods (30 min to 24 h). TXNIP mRNA was measured by quantitative real time PCR. HG significantly increases TXNIP mRNA expression when compared to LG. However, when the HG medium was changed to LG, TXNIP mRNA is reduced to the level of LG (without HG exposure) within 30 min and continues to repress up to 24 h. **(B).** Alternatively, we added IGF-1 (100 ng/ml) in HG medium and determined TXNIP mRNA expression at different times (30 min to 24 h). We found that IGF-1 decreases HG-induced TXNIP expression up to 4 h, then increases at 24 h. In fact, TXNIP mRNA rises at 6 h onwards after IGF-1 addition (not shown). These results suggest that TXNIP mRNA is relatively unstable and decay soon after withdrawal of its inducer, HG. Similarly, IGF-1 inhibition of HG-induced TXNIP mRNA expression also sustains, apparently, when IGF-1/IGF-1R signal persists (~up to 4-6h) [180]. The molecular mechanisms and signaling pathways involved in insulin/IGF-1 suppression of TXNIP under HG are yet to be determined. **(C)** HG also increases TXNIP in human retinal endothelial cells (HREC) and mouse microglia (BV2). The role of TXNIP in microglia oxidative stress and inflammation is yet to be determined.

TXNIP and pericyte apoptosis: An early hallmark of DR is the loss of capillary pericyte and formation of pericyte ghost or hypoperfused acellular capillaries in the retina [140-143]. The role of TXNIP in pericyte apoptosis in diabetes has not been investigated so far in *in vivo* models. Our *in vitro* studies under diabetic conditions using retinal PC showed that TXNIP is highly induced in PC by HG and causes MT dysfunction, ATP depletion, oxidative/nitrosative stress, DNA damage, and apoptosis [58]. Pericyte apoptosis under HG is prevented by treatment with anti-oxidant N-acetylcysteine or azaserine, an inhibitor of the glutamine fructose-6-phosphate transferase (GFAT), the rate limiting enzyme in the HBP, as well as TXNIP knock down by

siRNA. Furthermore, we have previously shown that renal mesangial cell, which is also involved in diabetic kidney disease, expresses TXNIP under HG and increased HBP flux causing aberrant extracellular matrix gene expression, oxidative stress, and apoptosis [70,144]. These effects are associated with changes in ER-stress and interferon-inducible gene expression as revealed the transcriptome profile in microarrays. On the other hand, TGF-β1 activates TXNIP early (2-4 h) in mesangial cells and then suppresses at 24 h [144]. IGF-1 down-regulates TXNIP expression in mesangial cells [144]. Similarly, PDGF down regulates TXNIP in smooth muscle cells and increases Trx activity [145]. PDGF-receptor beta is expressed in retinal pericyte and PDGF-B, produced by EC, is required for pericyte survival.

TXNIP and neuronal injury: TXNIP up-regulation in the diabetic rat retina correlates with cell death in GCL as revealed by caspase-3 activation [45] while TXNIP knock down by intravitreal siRNA prevents caspase-3 activation in GCL. The identity of cells undergoing apoptosis in the GCL is unknown at present whether they are retinal ganglions or displaced amacrine cells. In addition to GCL, caspase-3 staining is also seen at the interface of IPL and INL in diabetic rat retina where amacrine dopaminergic neurons and horizontal cells are located (Figure 1). TXNIP up-regulation parallels RGC death in glaucoma induced by optic nerve transaction or elevated intraocular pressure while Trx1 and Trx2 overexpression reduce RGC apoptosis [146]. Furthermore, Aβ-mediated retinal neurotoxicity in mice involves impairment of the thioredoxin system, which includes increases in TXNIP expression and oxidative stress [83]. This process may be related to decreases in TrxR1 activity as well as trapping of Trx by TXNIP [83]. In another model of retinal neurotoxicity in rats, intravitreal injection of NMDA induces severe RGC death, which is inhibited by co-treatment with verapamil to block TXNIP [81]. Inhibition of TXNIP also reduces Muller glia reactivity and NF-κB-mediated pro-inflammatory cytokine production [38]. We have also shown that, in partial sciatic nerve injury [111] and upper cervical spinal cord hemisection [147], TXNIP expression is significantly induced, which subsequently returns to the basal level after recovery. These results suggested a role for TXNIP in neuronal injury and recovery. Furthermore, TXNIP mRNA is induced in ischemic-reperfusion in the brain and hyperglycemia exacerbates oxidative stress and neuronal apoptotis [148]. It has also been shown that TXNIP expression increases in dorsal root ganglion in diabetic rats and causes oxidative stress by inhibiting Trx [71]. In this case, neither antioxidant R-lipoic acid nor p38 MAP kinase inhibitor SB239063 treatment reduces TXNIP expression in spite of reducing oxidative stress while insulin decreases Txnip expression. Recent studies also demonstrated that TXNIP is upregulated in the hypothalamus in mouse models of obesity and diabetes and it is implicated in nutrient sensing and energy metabolism [77,78]. Downregulation of mediobasal hypothalamic TXNIP expression prevents diet-induced obesity and insulin resistance. Together, these findings point to an important role of TXNIP in redox regulation, metabolism, and neuronal injury/death under various pathological conditions including DR.

TXNIP and glial activation: TXNIP up-regulation in DR correlates with radial GFAP expression (Figure 1) and pro-inflammatory cytokine IL-1β induction suggesting Muller glia reactivity (gliosis) [38,45]. That TXNIP is involved in glial activation is further demonstrated by suppression of GFAP and IL-1β expression in the diabetic retina after TXNIP knock down [38,111]. *In vitro*, HG causes sustained up-regulation of TXNIP, which results in ROS generation, ER-stress and autophagy/mitophagy in a rat Muller cell line, rMC1 [38]. Thus, a spatial and temporal cellular defense mechanism is activated in rMC1 by sustained HG, which include: (i) TXNIP up-regulation, (ii) pro-IL-1β induction, (iii) NLRP3 inflammasome and caspase-1 activation, (iv) ER stress response (sXBP1), (v) hypoxic-like HIF-1α stabilization, (vi) ROS/RNS stress and ATP reduction, and (vii) autophagy/mitophagy [38]. TXNIP ablation prevents HG-induced ROS generation, restores ATP level and autophagic LC3B (microtubule-associated light chain 3B) induction in rMC1. Similarly, HG and ATP induce TXNIP up-regulation and pro-IL-1β processing in rMC1 [82]. We also observed that HG increases TXNIP expression in a mouse microglial cell line, BV2 (Figure 5C), providing evidence that microglial activation may also contribute to IL-1β expression and secretion in DR. Thus, we and others have provided evidence that HG induces TXNIP up-regulation in the diabetic retina and plays a crucial role in pro-IL-1β induction and processing [38,82,110,149]. Nonetheless, mechanisms of TXNIP-induced NLRP3 inflammasome assembly ((containing NLRP3, ASC (apoptosis-associated speck-like protein) and pro-caspase-1)), caspase-1 activation and pro-IL-1β maturation are yet to be worked out [98,99,113,150,151]. The fact that TXNIP is involved in HG-induced pro-IL-1β priming (expression) makes is challenging to design experiments and demonstrate how TXNIP mediates pro-IL-1β processing and secretion using methods that blunt TXNIP itself. Furthermore, TXNIP is involved in NF-κB activation and NF-κB-mediated NLRP3 expression [59,149,152]. In any event, by blunting TXNIP, we demonstrated that pro-IL-1β expression and IL-1β secretion are inhibited [38,112]; therefore, TXNIP is a potential target to reduce retinal inflammation in DR.

TXNIP and RPE: A single layer of retinal RPE forms the outer BRB. RPE is involved in phagocytosis of the photoreceptor outer segment and visual pigment recycling. Nonetheless, the role of TXNIP in RPE function in DR is yet to be investigated. One study, investigating ceramide toxicity in human RPE, demonstrated TXNIP up-regulation by ceramide treatment [153]. Exogenous addition of recombinant Trx1 protected RPE cell death induced by ceramide, which is independent of the ASK-1 pathway. Recent studies have also shown NLRP3 inflammasome activation and IL-1β secretion in RPE cells under oxidative stress, implying RPE inflammation in geographic atrophy and neovascular age-related macular degeneration [154-156]. So far, studies of dyslipidemia in DR are limited [157-159] and the role of free fatty acids in TXNIP up-regulation and RPE dysfunction has not been investigated in diabetes. TXNIP expression is abundant in terminally differentiated epithelial cells in the colon and gastric and suggested critical role of redox regulation in epithelial differentiation and in mucosal immunity of the gastrointestinal tract [113,160]. Most retinal cells including the RPE are fully or terminally differentiated cells; therefore, TXNIP may play a critical role in retinal RPE metabolism, redox regulation, differentiation, and innate immune responses in diabetes.

Conclusion and Future Direction

TXNIP up-regulation, oxidative/nitrosative stress and putative duration-dependent cellular responses under chronic hyperglycemia (and/or frequent intermittent hyperglycemia) in the progression of DR are summarized in Figure 6. Initially, as hyperglycemia prevails in pre-diabetes/diabetes, the MT aerobic respiration will be enhanced via oxidative phosphorylation (OxPhoS) and ATP synthesis in the MT inner membrane electron transfer chain (ETC). Enhanced OxPhoS releases reactive oxygen radicals and increases ROS/RNS species, which eventually causes ROS/RNS stress, protein misfolding and ER-stress. A mild ER-stress inducing unfolded protein response may signal transcription of chaperones/heat shock proteins and anti-oxidant genes [161,162]. However, sustained ER-stress releases stored calcium,

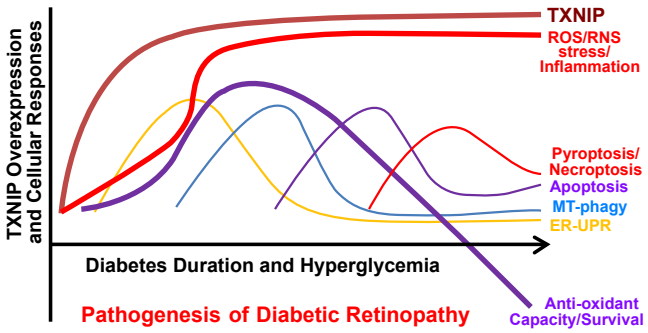

Figure 6: TXNIP overexpression and potential mechanisms for the pathogenesis of diabetic retinopathy. (i) Diabetes and hyperglycemia-induced TXNIP expression may initially be a stress response and protective event, which involves generation of a mild oxidative/nitrosative stress by trapping Trx. This process may induce anti-oxidant gene expression and reprogramming of glucose utilization by glycolysis and mitochondrial oxidative phosphorylation, which enhance the pentose phosphate pathway (PPP) and NADPH synthesis. Simultaneously, a mild ER-stress may be induced to activate er-UPR and anti-oxidant and chaperone synthesis. **(ii)** As hyperglycemia persists, TXNIP up-regulation sustains leading to ROS/RNS and er-stress. Stressed ER releases stored calcium, which is taken up by mitochondria, leading to mitochondrial dysfunction. Damaged mitochondria release ROS from its electron transfer in ETC and they are inefficient in ATP production. Mitophagy is thus activated to remove damaged mitochondria and maintain MT homeostasis as a survival mechanism. **(iii)** In addition, damaged mitochondria release cytochrome c and activate pro-apoptotic caspase-3. **(iv)** Nonetheless, apoptosis is an energy consuming process and requires MT ATP synthesis for packaging cytosolic and nuclear components by membrane vesicles. As the MT bioenergetics collapse and ATP is depleted, apoptosis gives way to necroptosis or pyroptosis. These latter cell death mechanisms involve plasma membrane leakage and release of cellular contents as DAMPs (damage associated molecular patterns). DAMPs are recognized by cytosolic and membrane bound pattern recognition receptors (PRRs) in innate immune cells (such as microglia, Muller glia, as well as by epithelial cells) as danger signals or alarmins, which evoke innate immune responses and inflammation. **(v)** As diabetes progresses and ROS/RNS stress prevails, the initial cellular anti-oxidant and chaperone capacity weaken and cell defensive mechanisms fail, a process known as hormesis – beneficial at low level and harmful at prolonged and higher level [162] - leading to disease progression of diabetic retinopathy. Therefore, TXNIP overexpression may play a critical role in cellular oxidative/nitrosative stress, inflammation, pre-mature cell death and progression of microvascular complications of diabetes including diabetic retinopathy. Thus, TXNIP represents a novel target for gene and drug therapies to prevent or slow down the progression of diabetic retinopathy.

which is taken up by mitochondria and activates calcium-dependent MT TCA cylce enzymes, NADH production, and ATP synthesis [163]. These processes further result in MT hyperpolarization, ROS production, membrane potential transition-pore opening, and subsequent depolarization. Repolatization of damaged MT membrane consumes energy (ATP) than it produces while damaged mitochondria release ROS. Therefore, dysfunctional mitochondria accumulate within the cell and evoke the process of autophagy/mitophagy to remove depolarized mitochondria by lysosomal degradation [105]. Furthermore, prolonged ER-stress inhibits global protein biosynthesis while damaged mitochondria releases cell death signals such as cytochrome c to the cytosol, which activates caspase-3 and apoptosis. Nonetheless, apoptotic processes require energy for packing nuclear and cytosolic components into membrane bound vesicles; therefore needed to maintain intact inner membrane ETC and ATP synthesis. Apoptosis is physiologically a non-inflammatory cell death path in which the fragmented cellular components are cleared by neighboring cells or by phagocytic immune cells such as microglia in the retina. However, when cellular energy level is low (usually less than 60% of the normal level) and mitochondria fail to produce ATP, then a pro-inflammatory cell death path of necroptosis or pyroptosis is evoked [164]. This later cell death involves plasma membrane leakage and released of nuclear and MT components as danger-associated molecular patterns (DAMPs) such as ATP, mtDNA, HSP60, HMGB1, IL-1β and others [165]. These DAMPS are recognized by membrane bound pattern recognition receptors (PRRs) including toll like receptors TLR2, TLR4, RAGE and by cytosolic TLR9, NLPR3, and AIM2. Thus, pro-inflammatory cytokines and chemokines (IL-1β, iNOS, COX-2, MCP1, VEGF-A and others) are secreted by innate immune cells primarily by retinal microglia, Muller cell, astrocyte as well as by RPE and EC that are detrimental for neuronal function and survival [3].

Acute or chronic CNS neuroinflammation is harmful because they cause irreversible neuronal damage and unresolved inflammation leading to premature cell death and perpetuate disease [3]. In pre-diabetes and early stages of diabetic retinopathy, hyperglycemia and metabolic abnormalities may establish a low-grade inflammatory environment, which favor metabolic switch from MT aerobic respiration to cytosolic anaerobic glycolysis, epigenetic adaptation and transcriptome reprogramming to the prevailing environment. Such adaptive processes may involve reactivation of mesenchymal genes involved in extracellular matrix remodeling and secretion of soluble factors thereby generating a vicious cycle of innate immunity, impaired wound healing (gliosis/fibrosis), cell death, and auto-inflammation [166]. Subsequently, cellular anti-oxidant capacity diminishes and cell viability is reduced. However, which of these cellular processes and which retinal cell type(s) represent best targets for ameliorating ocular complications of DR is still unclear and required further studies. Within an organ, different cells respond differently to external or internal stimuli based on their morphology and function. In case of the retina, PC and GCL neurons appear to be targets of early cell death in diabetes [38,45,58]. They seem to use MT respiration primarily as their energy source, which produces excess ROS under hyperglycemia.

Recently, it is becoming clear that epigenetics play a role in DR [59,167,168] and chromatin modification requires glucose metabolic products such as acetyl-CoA for HAT activity and histone acetylation and *S*-adenosylmethionine for histone and DNA methylation [169-172]. Epigenetic modifications once established are stable and heritable. Therefore, diabetes-induced metabolic imbalance, redox stress and MT defects could increase or decrease availability of these epigenetic substrates and reprogram chromatin configuration. It is currently understood that ninety percent of the genome are transcribed though only ~1.5% are protein coding mRNAs [171]. The role of these non-translated and non-coding RNAs, such as microRNA, piwi binding RNA, and long non-coding RNA, are critically involved in epigenetics and transcriptome maintenance under changing pathological conditions [171,173], specifically in fully or terminally differentiated cells that can't easily be renewed [174-176]. This aspect of ncRNAs in epigenetics and pathogenesis of DR will constitute an exciting new direction in future and is out of the preview of this article.

In late stage diabetes, insulin treatment or glycemic control may not completely reverse or normalize changes in retinal architecture and molecular abnormalities, a phenomenon described as glycemic or metabolic memory. Understanding epigenetic memory processes will provide clues to progression of DR after glucose normalization and, therefore, epigenetic drugs could become an important constituent of DR therapy. In addition, identification of early aberrant gene expression patterns and their regulatory mechanisms in DR, which lead to later clinical pathologies, will be critical for developing therapies to prevent or slow down ocular complications. In this regard, TXNIP

represents a novel gene target to reduce cellular oxidative/nitrosative stress, inflammation, and premature cell death in DR. Currently, TXNIP story is just the tip of the ice-berg in understanding metabolic deregulation in diabetes and its chronic complications including DR.

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References

1. Newman EA (2004) A dialogue between glia and neurons in the retina: modulation of neuronal excitability. Neuron Glia Biol 1: 245-252.
2. London A, Benhar I, Schwartz M (2013) The retina as a window to the brain-from eye research to CNS disorders. Nat Rev Neurol 9: 44-53.
3. Forrester JV, Xu H (2012) Good news-bad news: the Yin and Yang of immune privilege in the eye. Front Immunol 3: 338.
4. Frank RN, Turczyn TJ, Das A (1990) Pericyte coverage of retinal and cerebral capillaries. Invest Ophthalmol Vis Sci 31: 999-1007.
5. Daneman R, Zhou L, Kebede AA, Barres BA (2010) Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature 468: 562-566.
6. Hayden MR, Yang Y, Habibi J, Bagree SV, Sowers JR (2010) Pericytopathy: oxidative stress and impaired cellular longevity in the pancreas and skeletal muscle in metabolic syndrome and type 2 diabetes. Oxid Med Cell Longev 3: 290-303.
7. Am S, B P, Jj M (2013) Renal pericytes: multifunctional cells of the kidneys. Pflugers Arch 465: 767-773.
8. Liu H, Kim SY, Fu Y, Wu X, Ng L, et al. (2013) An isoform of retinoid-related orphan receptor Î² directs differentiation of retinal amacrine and horizontal interneurons. Nat Commun 4: 1813.
9. Wong-Riley MT (2010) Energy metabolism of the visual system. Eye Brain 2: 99-116.
10. Barnstable CJ (2009) Mitochondria and the regulation of free radical damage in the eye. J Ocul Biol Dis Infor 2: 145-148.
11. Santos JM, Tewari S, Kowluru RA (2012) A compensatory mechanism protects retinal mitochondria from initial insult in diabetic retinopathy. Free Radic Biol Med 53: 1729-1737.
12. Jarrett SG, Rohrer B, Perron NR, Beeson C, Boulton ME (2013) Assessment of mitochondrial damage in retinal cells and tissues using quantitative polymerase chain reaction for mitochondrial DNA damage and extracellular flux assay for mitochondrial respiration activity. Methods Mol Biol 935: 227-243.
13. Koushan K, Rusovici R, Li W, Ferguson LR, Chalam KV (2013) The role of lutein in eye-related disease. Nutrients 5: 1823-1839.
14. Penn JS, Madan A, Caldwell RB, Bartoli M, Caldwell RW, et al. (2008) Vascular endothelial growth factor in eye disease. Prog Retin Eye Res 27: 331-371.
15. Feng Y, Busch S, Gretz N, Hoffmann S, Hammes HP (2012) Crosstalk in the retinal neurovascular unit - lessons for the diabetic retina. Exp Clin Endocrinol Diabetes 120: 199-201.
16. Pournaras CJ, Rungger-Brändle E, Riva CE, Hardarson SH, Stefansson E (2008) Regulation of retinal blood flow in health and disease. Prog Retin Eye Res 27: 284-330.
17. Reichenbach A, Bringmann A (2013) New functions of Müller cells. Glia 61: 651-678.
18. Zhao M, Valamanesh F, Celerier I, Savoldelli M, Jonet L, et al. (2010) The neuroretina is a novel mineralocorticoid target: aldosterone up-regulates ion and water channels in Müller glial cells. FASEB J 24: 3405-3415.
19. Zhao M, Bousquet E, Valamanesh F, Farman N, Jeanny JC, et al. (2011) Differential regulations of AQP4 and Kir4.1 by triamcinolone acetonide and dexamethasone in the healthy and inflamed retina. Invest Ophthalmol Vis Sci 52: 6340-6347.
20. Ball AK, McReynolds JS (1998) Localization of gap junctions and tracer coupling in retinal Müller cells. J Comp Neurol 393: 48-57.
21. Zahs KR, Kofuji P, Meier C, Dermietzel R (2003) Connexin immunoreactivity in glial cells of the rat retina. J Comp Neurol 455: 531-546.
22. Zahs KR, Ceelen PW (2006) Gap junctional coupling and connexin immunoreactivity in rabbit retinal glia. Vis Neurosci 23: 1-10.
23. Gonzalez-Rey E, Chorny A, Delgado M (2007) Regulation of immune tolerance by anti-inflammatory neuropeptides. Nat Rev Immunol 7: 52-63.
24. Sugita S (2009) Role of ocular pigment epithelial cells in immune privilege. Arch Immunol Ther Exp (Warsz) 57: 263-268.
25. Sugita S, Horie S, Yamada Y, Kawazoe Y, Takase H, et al. (2011) Suppression of interleukin-17-producing T-helper 17 cells by retinal pigment epithelial cells. Jpn J Ophthalmol 55: 565-575.
26. Tu Z, Li Y, Smith DS, Sheibani N, Huang S, et al. (2011) Retinal pericytes inhibit activated T cell proliferation. Invest Ophthalmol Vis Sci 52: 9005-9010.
27. Giunta S, Castorina A, Bucolo C, Magro G, Drago F, et al. (2012) Early changes in pituitary adenylate cyclase-activating peptide, vasoactive intestinal peptide and related receptors expression in retina of streptozotocin-induced diabetic rats. Peptides 37: 32-39.
28. Dyer MA, Cepko CL (2000) Control of Müller glial cell proliferation and activation following retinal injury. Nat Neurosci 3: 873-880.
29. Levine EM, Close J, Fero M, Ostrovsky A, Reh TA (2000) p27(Kip1) regulates cell cycle withdrawal of late multipotent progenitor cells in the mammalian retina. Dev Biol 219: 299-314.
30. Lee TC, Almeida D, Claros N, Abramson DH, Cobrinik D (2006) Cell cycle-specific and cell type-specific expression of Rb in the developing human retina. Invest Ophthalmol Vis Sci 47: 5590-5598.
31. Pacal M, Bremner R (2012) Mapping differentiation kinetics in the mouse retina reveals an extensive period of cell cycle protein expression in post-mitotic newborn neurons. Dev Dyn 241: 1525-1544.
32. Pattwell DM, Stappler T, Sheridan C, Heimann H, Gibran SK, et al. (2010) Fibrous membranes in diabetic retinopathy and bevacizumab. Retina 30: 1012-1016.
33. Liu Y (2011) Cellular and molecular mechanisms of renal fibrosis. Nat Rev Nephrol 7: 684-696.
34. Hasturk H, Kantarci A, Van Dyke TE (2012) Oral inflammatory diseases and systemic inflammation: role of the macrophage. Front Immunol 3: 118.
35. Nishikawa T, Kukidome D, Sonoda K, Fujisawa K, Matsuhisa T, et al. (2007) Impact of mitochondrial ROS production on diabetic vascular complications. Diabetes Res Clin Pract 77 Suppl 1:S41-S45.
36. Oshitari T, Hata N, Yamamoto S (2008) Endoplasmic reticulum stress and diabetic retinopathy. Vasc Health Risk Manag 4: 115-122.
37. Barot M, Gokulgandhi MR, Mitra AK (2011) Mitochondrial dysfunction in retinal diseases. Curr Eye Res 36: 1069-1077.
38. Devi TS, Lee I, Hüttemann M, Kumar A, Nantwi KD, et al. (2012) TXNIP links innate host defense mechanisms to oxidative stress and inflammation in retinal Muller glia under chronic hyperglycemia: implications for diabetic retinopathy. Exp Diabetes Res 2012: 438238.
39. Roy S, Trudeau K, Roy S, Tien T, Barrette KF (2012) Mitochondrial Dysfunction and Endoplasmic Reticulum Stress in Diabetic Retinopathy: A Mechanistic Insight for High Glucose-Induced Retinal Cell Death. Curr Clin Pharmacol.
40. Zhong Y, Li J, Chen Y, Wang JJ, Ratan R, et al. (2012) Activation of endoplasmic reticulum stress by hyperglycemia is essential for Müller cell-derived inflammatory cytokine production in diabetes. Diabetes 61: 492-504.
41. Bringmann A, Iandiev I, Pannicke T, Wurm A, Hollborn M, et al. (2009) Cellular signaling and factors involved in Müller cell gliosis: neuroprotective and detrimental effects. Prog Retin Eye Res 28: 423-451.
42. Rodríguez JJ, Verkhratsky A (2011) Neuroglial roots of neurodegenerative diseases? Mol Neurobiol 43: 87-96.
43. Gardiner TA, Anderson HR, Stitt AW (2003) Inhibition of advanced glycation end-products protects against retinal capillary basement membrane expansion during long-term diabetes. J Pathol 201: 328-333.

44. Cherian S, Roy S, Pinheiro A, Roy S (2009) Tight glycemic control regulates fibronectin expression and basement membrane thickening in retinal and glomerular capillaries of diabetic rats. Invest Ophthalmol Vis Sci 50: 943-949.

45. Perrone L, Devi TS, Hosoya KI, Terasaki T, Singh LP (2010) Inhibition of TXNIP expression in vivo blocks early pathologies of diabetic retinopathy. Cell Death Dis 1: e65.

46. Devi TS, Singh LP, Hosoya K, Terasaki T (2011) GSK-3ß/CREB axis mediates IGF-1-induced ECM/adhesion molecule expression, cell cycle progression and monolayer permeability in retinal capillary endothelial cells: Implications for diabetic retinopathy. Biochim Biophys Acta 1812: 1080-1088.

47. Frey T, Antonetti DA (2011) Alterations to the blood-retinal barrier in diabetes: cytokines and reactive oxygen species. Antioxid Redox Signal 15: 1271-1284.

48. Rangasamy S, Srinivasan R, Maestas J, McGuire PG, Das A (2011) A potential role for angiopoietin 2 in the regulation of the blood-retinal barrier in diabetic retinopathy. Invest Ophthalmol Vis Sci 52: 3784-3791.

49. Jarajapu YP, Cai J, Yan Y, Li Calzi S, Kielczewski JL, et al. (2012) Protection of blood retinal barrier and systemic vasculature by insulin-like growth factor binding protein-3. PLoS One 7: e39398.

50. Simó R, Villarroel M, Corraliza L, Hernández C, Garcia-Ramírez M (2010) The retinal pigment epithelium: something more than a constituent of the blood-retinal barrier--implications for the pathogenesis of diabetic retinopathy. J Biomed Biotechnol 2010: 190724.

51. Campbell M, Humphries P (2012) The blood-retina barrier: tight junctions and barrier modulation. Adv Exp Med Biol 763: 70-84.

52. Ryu JK, McLarnon JG (2009) A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. J Cell Mol Med 13: 2911-2925.

53. Davalos D, Ryu JK, Merlini M, Baeten KM, Le Moan N, et al. (2012) Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nat Commun 3: 1227.

54. Bringmann A, Wiedemann P (2012) Müller glial cells in retinal disease. Ophthalmologica 227: 1-19.

55. Kowluru RA, Kanwar M, Kennedy A (2007) Metabolic memory phenomenon and accumulation of peroxynitrite in retinal capillaries. Exp Diabetes Res 2007: 21976.

56. Chan PS, Kanwar M, Kowluru RA (2010) Resistance of retinal inflammatory mediators to suppress after reinstitution of good glycemic control: novel mechanism for metabolic memory. J Diabetes Complications 24: 55-63.

57. Santos JM, Kowluru RA (2011) Role of mitochondria biogenesis in the metabolic memory associated with the continued progression of diabetic retinopathy and its regulation by lipoic acid. Invest Ophthalmol Vis Sci 52: 8791-8798.

58. Devi TS, Hosoya K, Terasaki T, Singh LP (2013) Critical role of TXNIP in oxidative stress, DNA damage and retinal pericyte apoptosis under high glucose: implications for diabetic retinopathy. Exp Cell Res 319: 1001-1012.

59. Perrone L, Devi TS, Hosoya K, Terasaki T, Singh LP (2009) Thioredoxin interacting protein (TXNIP) induces inflammation through chromatin modification in retinal capillary endothelial cells under diabetic conditions. J Cell Physiol 221: 262-272.

60. Chen KS, DeLuca HF (1994) Isolation and characterization of a novel cDNA from HL-60 cells treated with 1,25-dihydroxyvitamin D-3. Biochim Biophys Acta 1219: 26-32.

61. Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H, et al. (1999) Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. J Biol Chem 274: 21645-21650.

62. Han SH, Jeon JH, Ju HR, Jung U, Kim KY, et al. (2003) VDUP1 upregulated by TGF-beta1 and 1,25-dihydorxyvitamin D3 inhibits tumor cell growth by blocking cell-cycle progression. Oncogene 22: 4035-4046.

63. Nishinaka Y, Nishiyama A, Masutani H, Oka S, Ahsan KM, et al. (2004) Loss of thioredoxin-binding protein-2/vitamin D3 up-regulated protein 1 in human T-cell leukemia virus type I-dependent T-cell transformation: implications for adult T-cell leukemia leukemogenesis. Cancer Res 64: 1287-1292.

64. Ahsan MK, Masutani H, Yamaguchi Y, Kim YC, Nosaka K, et al. (2006) Loss of interleukin-2-dependency in HTLV-I-infected T cells on gene silencing of thioredoxin-binding protein-2. Oncogene 25: 2181-2191.

65. Bodnar JS, Chatterjee A, Castellani LW, Ross DA, Ohmen J, et al. (2002) Positional cloning of the combined hyperlipidemia gene Hyplip1. Nat Genet 30: 110-116.

66. Pajukanta P, Lilja HE, Sinsheimer JS, Cantor RM, Lusis AJ, et al. (2004) Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). Nat Genet 36: 371-376.

67. Coon H, Xin Y, Hopkins PN, Cawthon RM, Hasstedt SJ, et al. (2005) Upstream stimulatory factor 1 associated with familial combined hyperlipidemia, LDL cholesterol, and triglycerides. Hum Genet 117: 444-451.

68. Shalev A, Pise-Masison CA, Radonovich M, Hoffmann SC, Hirshberg B, et al. (2002) Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGF-beta signaling pathway. Endocrinology 143: 3695-3698.

69. Kobayashi T, Uehara S, Ikeda T, Itadani H, Kotani H (2003) Vitamin D3 up-regulated protein-1 regulates collagen expression in mesangial cells. Kidney Int 64: 1632-1642.

70. Cheng DW, Jiang Y, Shalev A, Kowluru R, Crook ED, et al. (2006) An analysis of high glucose and glucosamine-induced gene expression and oxidative stress in renal mesangial cells. Arch Physiol Biochem 112: 189-218.

71. Price SA, Gardiner NJ, Duran-Jimenez B, Zeef LA, Obrosova IG, et al. (2006) Thioredoxin interacting protein is increased in sensory neurons in experimental diabetes. Brain Res 1116: 206-214.

72. Minn AH, Hafele C, Shalev A (2005) Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. Endocrinology 146: 2397-2405.

73. Chen J, Saxena G, Mungrue IN, Lusis AJ, Shalev A (2008) Thioredoxin-interacting protein: a critical link between glucose toxicity and beta-cell apoptosis. Diabetes 57: 938-944.

74. Saxena G, Chen J, Shalev A (2010) Intracellular shuttling and mitochondrial function of thioredoxin-interacting protein. J Biol Chem 285: 3997-4005.

75. Masson E, Koren S, Razik F, Goldberg H, Kwan EP, et al. (2009) High beta-cell mass prevents streptozotocin-induced diabetes in thioredoxin-interacting protein-deficient mice. Am J Physiol Endocrinol Metab 296: E1251-E1261.

76. Stoltzman CA, Peterson CW, Breen KT, Muoio DM, Billin AN, Ayer DE (2008) Glucose sensing by MondoA: Mlx complexes: a role for hexokinases and direct regulation of thioredoxin-interacting protein expression. Proc Natl Acad Sci U S A 105: 6912-6917.

77. Blouet C, Schwartz GJ (2011) Nutrient-sensing hypothalamic TXNIP links nutrient excess to energy imbalance in mice. J Neurosci 31: 6019-6027.

78. Blouet C, Liu SM, Jo YH, Chua S, Schwartz GJ (2012) TXNIP in Agrp neurons regulates adiposity, energy expenditure, and central leptin sensitivity. J Neurosci 32: 9870-9877.

79. Bixler GV, Vanguilder HD, Brucklacher RM, Kimball SR, Bronson SK, et al. (2011) Chronic insulin treatment of diabetes does not fully normalize alterations in the retinal transcriptome. BMC Med Genomics 4: 40.

80. Munemasa Y, Ahn JH, Kwong JM, Caprioli J, Piri N (2009) Redox proteins thioredoxin 1 and thioredoxin 2 support retinal ganglion cell survival in experimental glaucoma. Gene Ther 16: 17-25.

81. Al-Gayyar MM, Abdelsaid MA, Matragoon S, Pillai BA, El-Remessy AB (2011) Thioredoxin interacting protein is a novel mediator of retinal inflammation and neurotoxicity. Br J Pharmacol 164: 170-180.

82. Trueblood KE, Mohr S, Dubyak GR (2011) Purinergic regulation of high-glucose-induced caspase-1 activation in the rat retinal Müller cell line rMC-1. Am J Physiol Cell Physiol 301: C1213-1223.

83. Lamoke F, Ripandelli G, Webster S, Montemari A, Maraschi A, et al. (2012) Loss of thioredoxin function in retinas of mice overexpressing amyloid Î². Free Radic Biol Med 53: 577-588.

84. Levendusky MC, Basle J, Chang S, Mandalaywala NV, Voigt JM, et al. (2009) Expression and regulation of vitamin D3 upregulated protein 1 (VDUP1) is conserved in mammalian and insect brain. J Comp Neurol 517: 581-600.

85. Wang Z, Rong YP, Malone MH, Davis MC, Zhong F, et al. (2006) Thioredoxin-interacting protein (txnip) is a glucocorticoid-regulated primary response gene involved in mediating glucocorticoid-induced apoptosis. Oncogene 25: 1903-1913.

86. Reich E, Tamary A, Sionov RV, Melloul D (2012) Involvement of thioredoxin-interacting protein (TXNIP) in glucocorticoid-mediated beta cell death. Diabetologia 55: 1048-1057.

87. de Candia P, Blekhman R, Chabot AE, Oshlack A, Gilad Y (2008) A combination of genomic approaches reveals the role of FOXO1a in regulating an oxidative stress response pathway. PLoS One 3: e1670.

88. Al-Mubarak B, Soriano FX, Hardingham GE (2009) Synaptic NMDAR activity suppresses FOXO1 expression via a cis-acting FOXO binding site: FOXO1 is a FOXO target gene. Channels (Austin) 3: 233-238.

89. Li X, Rong Y, Zhang M, Wang XL, LeMaire SA, et al. (2009) Up-regulation of thioredoxin interacting protein (Txnip) by p38 MAPK and FOXO1 contributes to the impaired thioredoxin activity and increased ROS in glucose-treated endothelial cells. Biochem Biophys Res Commun 381: 660-665.

90. He X, Ma Q (2012) Redox regulation by nuclear factor erythroid 2-related factor 2: gatekeeping for the basal and diabetes-induced expression of thioredoxin-interacting protein. Mol Pharmacol 82: 887-897.

91. Minn AH, Couto FM, Shalev A (2006) Metabolism-independent sugar effects on gene transcription: the role of 3-O-methylglucose. Biochemistry 45: 11047-11051.

92. Stoltzman CA, Kaadige MR, Peterson CW, Ayer DE (2011) MondoA senses non-glucose sugars: regulation of thioredoxin-interacting protein (TXNIP) and the hexose transport curb. J Biol Chem 286: 38027-38034.

93. Yu FX, Goh SR, Dai RP, Luo Y (2009) Adenosine-containing molecules amplify glucose signaling and enhance txnip expression. Mol Endocrinol 23: 932-942.

94. Lekva T, Bollerslev J, Sahraoui A, Scholz H, Bøyum H, et al. (2013) Thioredoxin Interacting Protein Is a Potential Regulator of Glucose and Energy Homeostasis in Endogenous Cushing's Syndrome. PLoS One 8: e64247.

95. Shao W, Yu Z, Fantus IG, Jin T (2010) Cyclic AMP signaling stimulates proteasome degradation of thioredoxin interacting protein (TxNIP) in pancreatic beta-cells. Cell Signal 22: 1240-1246.

96. Chen J, Cha-Molstad H, Szabo A, Shalev A (2009) Diabetes induces and calcium channel blockers prevent cardiac expression of proapoptotic thioredoxin-interacting protein. Am J Physiol Endocrinol Metab 296: E1133-1139.

97. Wu N, Zheng B, Shaywitz A, Dagon Y, Tower C, et al. (2013) AMPK-dependent degradation of TXNIP upon energy stress leads to enhanced glucose uptake via GLUT1. Mol Cell 49: 1167-1175.

98. Lerner AG, Upton JP, Praveen PV, Ghosh R, Nakagawa Y, et al. (2012) IRE1Î± induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. Cell Metab 16: 250-264.

99. Oslowski CM, Hara T, O'Sullivan-Murphy B, Kanekura K, Lu S, et al. (2012) Thioredoxin-interacting protein mediates ER stress-induced Î² cell death through initiation of the inflammasome. Cell Metab 16: 265-273.

100. Benhar M, Forrester MT, Stamler JS (2006) Nitrosative stress in the ER: a new role for S-nitrosylation in neurodegenerative diseases. ACS Chem Biol 1: 355-358.

101. Chen X, Zhang X, Li C, Guan T, Shang H, et al. (2013) S-nitrosylated protein disulfide isomerase contributes to mutant SOD1 aggregates in amyotrophic lateral sclerosis. J Neurochem 124: 45-58.

102. Jin HO, Seo SK, Kim YS, Woo SH, Lee KH, et al. (2011) TXNIP potentiates Redd1-induced mTOR suppression through stabilization of Redd1. Oncogene 30: 3792-3801.

103. Zhou R, Yazdi AS, Menu P, Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. Nature 469: 221-225.

104. Rodgers MA, Bowman JW, Liang Q, Jung JU (2013) Regulation where autophagy intersects the inflammasome. Antioxid Redox Signal.

105. Liesa M, Shirihai OS (2013) Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. Cell Metab 17: 491-506.

106. Meuillet EJ, Mahadevan D, Berggren M, Coon A, Powis G (2004) Thioredoxin-1 binds to the C2 domain of PTEN inhibiting PTEN's lipid phosphatase activity and membrane binding: a mechanism for the functional loss of PTEN's tumor suppressor activity. Arch Biochem Biophys 429: 123-133.

107. Hers I, Vincent EE, Tavaré JM (2011) Akt signalling in health and disease. Cell Signal 23: 1515-1527.

108. Chen Y, Dorn GW 2nd (2013) PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science 340: 471-475.

109. Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, et al. (2013) Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. Nature 496: 372-376.

110. Kang R, Livesey KM, Zeh HJ 3rd, Lotze MT, Tang D (2011) Metabolic regulation by HMGB1-mediated autophagy and mitophagy. Autophagy 7: 1256-1258.

111. Li G, Liang X, Lotze MT (2013) HMGB1: The Central Cytokine for All Lymphoid Cells. Front Immunol 4: 68.

112. Sbai O, Devi TS, Melone MA, Feron F, Khrestchatisky M, et al. (2010) RAGE-TXNIP axis is required for S100B-promoted Schwann cell migration, fibronectin expression and cytokine secretion. J Cell Sci 123: 4332-4339.

113. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J (2010) Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 11: 136-140.

114. Yang H, Lundbäck P, Ottosson L, Erlandsson-Harris H, Venereau E, et al. (2012) Redox modification of cysteine residues regulates the cytokine activity of high mobility group box-1 (HMGB1). Mol Med 18: 250-259.

115. Yang H, Antoine DJ, Andersson U, Tracey KJ (2013) The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. J Leukoc Biol 93: 865-873.

116. Go YM, Jones DP (2013) Thiol/disulfide redox states in signaling and sensing. Crit Rev Biochem Mol Biol 48: 173-181.

117. Wang P, Liu GH, Wu K, Qu J, Huang B, et al. (2009) Repression of classical nuclear export by S-nitrosylation of CRM1. J Cell Sci 122: 3772-3779.

118. Sha Y, Marshall HE (2012) S-nitrosylation in the regulation of gene transcription. Biochim Biophys Acta 1820: 701-711.

119. Um HC, Jang JH, Kim DH, Lee C, Surh YJ (2011) Nitric oxide activates Nrf2 through S-nitrosylation of Keap1 in PC12 cells. Nitric Oxide 25: 161-168.

120. Koriyama Y, Kamiya M, Takadera T, Arai K, Sugitani K, et al. (2012) Protective action of nipradilol mediated through S-nitrosylation of Keap1 and HO-1 induction in retinal ganglion cells. Neurochem Int 61: 1242-1253.

121. Tomoda K, Kubota Y, Kato J (1999) Degradation of the cyclin-dependent-kinase inhibitor p27Kip1 is instigated by Jab1. Nature 398: 160-165.

122. Cheng X, Zhou Z, Xu G, Zhao J, Wu H, et al. (2013) Dynamic Changes of Jab1 and p27kip1 Expression in Injured Rat Sciatic Nerve. J Mol Neurosci.

123. Jeon JH, Lee KN, Hwang CY, Kwon KS, You KH, et al. (2005) Tumor suppressor VDUP1 increases p27(kip1) stability by inhibiting JAB1. Cancer Res 65: 4485-4489.

124. Hwang CY, Ryu YS, Chung MS, Kim KD, Park SS, et al. (2004) Thioredoxin modulates activator protein 1 (AP-1) activity and p27Kip1 degradation through direct interaction with Jab1. Oncogene 23: 8868-8875.

125. Chutkow WA, Birkenfeld AL, Brown JD, Lee HY, Frederick DW, et al. (2010) Deletion of the alpha-arrestin protein Txnip in mice promotes adiposity and adipogenesis while preserving insulin sensitivity. Diabetes 59: 1424-1434.

126. Patwari P, Lee RT (2012) An expanded family of arrestins regulate metabolism. Trends Endocrinol Metab 23: 216-222.

127. Patwari P, Chutkow WA, Cummings K, Verstraeten VL, Lammerding J, et al. (2009) Thioredoxin-independent regulation of metabolism by the alpha-arrestin proteins. J Biol Chem 284: 24996-25003.

128. World C, Spindel ON, Berk BC (2011) Arterioscler Thromb Vasc Biol. Thioredoxin-interacting protein mediates TRX1 translocation to the plasma membrane in response to tumor necrosis factor-a: a key mechanism for vascular endothelial growth factor receptor-2 transactivation by reactive oxygen species. Arterioscler Thromb Vasc Biol 31: 1890-1897.

129. Spindel ON, Yan C, Berk BC (2012) Thioredoxin-interacting protein mediates nuclear-to-plasma membrane communication: role in vascular endothelial growth factor 2 signaling. Arterioscler Thromb Vasc Biol 32: 1264-1270.

130. El-Remessy AB, Abdelsaid MA, Matragoon S (2013) Thioredoxin Interacting Protein (TXNIP) expression is required for VEGF-mediated angiogenic signal in endothelial cells. Antioxid Redox Signal.

131. Yamawaki H, Pan S, Lee RT, Berk BC (2005) Fluid shear stress inhibits

vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. J Clin Invest 115: 733-738.

132. Wang XQ, Nigro P, World C, Fujiwara K, Yan C, et al. (2012) Thioredoxin interacting protein promotes endothelial cell inflammation in response to disturbed flow by increasing leukocyte adhesion and repressing Kruppel-like factor 2. Circ Res 110: 560-568.

133. Marrachelli VG, Miranda FJ, Centeno JM, Salom JB, Torregrosa G, et al. (2010) Role of NO-synthases and cyclooxygenases in the hyperreactivity of male rabbit carotid artery to testosterone under experimental diabetes. Pharmacol Res 61: 62-70.

134. Misfeldt MW, Pedersen SM, Bek T (2013) Perivascular cells with pericyte characteristics are involved in ATP- and PGE(2)-induced relaxation of porcine retinal arterioles in vitro. Invest Ophthalmol Vis Sci 54: 3258-3264.

135. Ganesan P, He S, Xu H (2010) Analysis of retinal circulation using an image-based network model of retinal vasculature. Microvasc Res 80: 99-109.

136. Antonetti DA, Klein R, Gardner TW (2012) Diabetic retinopathy. N Engl J Med 366: 1227-1239.

137. Frank RN (2009) Metabolic memory in diabetes is true long-term memory. Arch Ophthalmol 127: 330-331.

138. Zhang L, Chen B, Tang L (2012) Metabolic memory: mechanisms and implications for diabetic retinopathy. Diabetes Res Clin Pract 96: 286-293.

139. Tang J, Du Y, Petrash JM, Sheibani N, Kern TS (2013) Deletion of aldose reductase from mice inhibits diabetes-induced retinal capillary degeneration and superoxide generation. PLoS One 8: e62081.

140. Hammes HP, Lin J, Renner O, Shani M, Lundqvist A, et al. (2002) Pericytes and the pathogenesis of diabetic retinopathy. Diabetes 51: 3107-3112.

141. Kobayashi T, Puro DG (2007) Loss of insulin-mediated vasoprotection: early effect of diabetes on pericyte-containing microvessels of the retina. Invest Ophthalmol Vis Sci 48: 2350-2355.

142. Geraldes P, Hiraoka-Yamamoto J, Matsumoto M, Clermont A, Leitges M, et al. (2009) Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. Nat Med 15: 1298-1306.

143. Fu D, Wu M, Zhang J, Du M, Yang S, et al. (2012) Mechanisms of modified LDL-induced pericyte loss and retinal injury in diabetic retinopathy. Diabetologia 55: 3128-3140.

144. Cheng, DW, Y Jiang, Crook ED, Singh LP (2007) Transforming growth factor b1-mediated mRNA expression and oxidative stress in renal mesangial cells: Comparison with high glucose and hexosamine -induced gene expression profiles. Biochemistry – An Indian Journal 1: 68-82.

145. Schulze PC, De Keulenaer GW, Yoshioka J, Kassik KA, Lee RT (2002) Vitamin D3-upregulated protein-1 (VDUP-1) regulates redox-dependent vascular smooth muscle cell proliferation through interaction with thioredoxin. Circ Res 91: 689-695.

146. Munemasa Y, Kwong JM, Kim SH, Ahn JH, Caprioli J, et al. (2010) Thioredoxins 1 and 2 protect retinal ganglion cells from pharmacologically induced oxidative stress, optic nerve transection and ocular hypertension. Adv Exp Med Biol 664: 355-363.

147. Singh LP, Devi TS, Nantwi KD (2012) Theophylline regulates inflammatory and neurotrophic factor signals in functional recovery after C2-hemisection in adult rats. Exp Neurol 238: 79-88.

148. Kim GS, Jung JE, Narasimhan P, Sakata H, Chan PH (2012) Induction of thioredoxin-interacting protein is mediated by oxidative stress, calcium, and glucose after brain injury in mice. Neurobiol Dis 46: 440-449.

149. Koenen TB, Stienstra R, van Tits LJ, de Graaf J, Stalenhoef AF, et al. (2011) Hyperglycemia activates caspase-1 and TXNIP-mediated IL-1beta transcription in human adipose tissue. Diabetes 60: 517-524.

150. Lunov O, Syrovets T, Loos C, Nienhaus GU, Mailänder V, et al. (2011) Amino-functionalized polystyrene nanoparticles activate the NLRP3 inflammasome in human macrophages. ACS Nano 5: 9648-9657.

151. Masters SL, Dunne A, Subramanian SL, Hull RL, Tannahill GM, et al. (2010) Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1Î² in type 2 diabetes. Nat Immunol 11: 897-904.

152. Qiao Y, Wang P, Qi J, Zhang L, Gao C (2012) TLR-induced NF-Î°B activation regulates NLRP3 expression in murine macrophages. FEBS Lett 586: 1022-1026.

153. Sreekumar PG, Ding Y, Ryan SJ, Kannan R, Hinton DR (2009) Regulation of thioredoxin by ceramide in retinal pigment epithelial cells. Exp Eye Res 88: 410-417.

154. Kauppinen A, Niskanen H, Suuronen T, Kinnunen K, Salminen A, et al. (2012) Oxidative stress activates NLRP3 inflammasomes in ARPE-19 cells--implications for age-related macular degeneration (AMD). Immunol Lett 147: 29-33.

155. Tarallo V, Hirano Y, Gelfand BD, Dridi S, Kerur N, et al. (2012) DICER1 loss and Alu RNA induce age-related macular degeneration via the NLRP3 inflammasome and MyD88. Cell 149: 847-859.

156. Tseng WA, Thein T, Kinnunen K, Lashkari K, Gregory MS, et al. (2013) NLRP3 inflammasome activation in retinal pigment epithelial cells by lysosomal destabilization: implications for age-related macular degeneration. Invest Ophthalmol Vis Sci 54: 110-120.

157. Chen W, Jump DB, Grant MB, Esselman WJ, Busik JV (2003) Dyslipidemia, but not hyperglycemia, induces inflammatory adhesion molecules in human retinal vascular endothelial cells. Invest Ophthalmol Vis Sci 44: 5016-5022.

158. Okuyama H, Yoshida T, Son A, Oka S, Wang D, et al. (2009) Thioredoxin binding protein 2 modulates natural killer T cell-dependent innate immunity in the liver: possible link to lipid metabolism. Antioxid Redox Signal 11: 2585-2593.

159. Busik JV, Esselman WJ, Reid GE (2012) Examining the role of lipid mediators in diabetic retinopathy. Clin Lipidol 7: 661-675.

160. Shao Y, Kim SY, Shin D, Kim MS, Suh HW, et al. (2010) TXNIP regulates germinal center generation by suppressing BCL-6 expression. Immunol Lett 129: 78-84.

161. Vendelbo MH, Nair KS (2011) Mitochondrial longevity pathways. Biochim Biophys Acta 1813: 634-644.

162. Cornelius C, Perrotta R, Graziano A, Calabrese EJ, Calabrese V (2013) Stress responses, vitagenes and hormesis as critical determinants in aging and longevity: Mitochondria as a “chi”. Immun Ageing 10: 15.

163. Carafoli E (2012) The interplay of mitochondria with calcium: an historical appraisal. Cell Calcium 52: 1-8.

164. Kaczmarek A, Vandenabeele P, Krysko DV (2013) Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. Immunity 38: 209-223.

165. Krysko DV, Agostinis P, Krysko O, Garg AD, Bachert C, et al. (2011) Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation. Trends Immunol 32: 157-164.

166. Nagata S, Kawane K (2011) Autoinflammation by endogenous DNA. Adv Immunol 110: 139-161.

167. Zhong Q, Kowluru RA (2013) Epigenetic modification of Sod2 in the development of diabetic retinopathy and in the metabolic memory: role of histone methylation. Invest Ophthalmol Vis Sci 54: 244-250.

168. He S, Li X, Chan N, Hinton DR (2013) Review: Epigenetic mechanisms in ocular disease. Mol Vis 19: 665-674.

169. Kaelin WG Jr, McKnight SL (2013) Influence of metabolism on epigenetics and disease. Cell 153: 56-69.

170. Masri S, Patel VR, Eckel-Mahan KL, Peleg S, Forne I, et al. (2013) Circadian acetylome reveals regulation of mitochondrial metabolic pathways. Proc Natl Acad Sci U S A 110: 3339-3344.

171. Blelloch R, Gutkind JS (2013) Epigenetics, noncoding RNAs, and cell signaling--crossroads in the regulation of cell fate decisions. Curr Opin Cell Biol 25: 149-151.

172. Shyh-Chang N, Locasale JW, Lyssiotis CA, Zheng Y, Teo RY, et al. (2013) Influence of threonine metabolism on S-adenosylmethionine and histone methylation. Science 339: 222-226.

173. Kanduri C (2011) Long noncoding RNA and epigenomics. Adv Exp Med Biol 722: 174-195.

174. Bian S, Sun T (2011) Functions of noncoding RNAs in neural development and neurological diseases. Mol Neurobiol 44: 359-373.

175. Sun AX, Crabtree GR, Yoo AS (2013) MicroRNAs: regulators of neuronal fate. Curr Opin Cell Biol 25: 215-221.

176. Meola N, Pizzo M, Alfano G, Surace EM, Banfi S (2012) The long noncoding RNA Vax2os1 controls the cell cycle progression of photoreceptor progenitors in the mouse retina. RNA 18: 111-123.

177. Schulze PC, Yoshioka J, Takahashi T, He Z, King GL, et al. (2004) Hyperglycemia promotes oxidative stress through inhibition of thioredoxin function by thioredoxin-interacting protein. Hyperglycemia promotes oxidative stress through inhibition of thioredoxin function by thioredoxin-interacting protein. J Biol Chem 279: 30369-30374.

178. Forrester MT, Seth D, Hausladen A, Eyler CE, Foster MW, et al. (2009) Thioredoxin-interacting protein (Txnip) is a feedback regulator of S-nitrosylation. J Biol Chem 284: 36160-36166.

179. Klionsky D (2013) An overview of autophagy: Morphology, mechanism and regulation. Antioxid Redox Signal.

180. Singh LP, Jiang Y, Cheng DW (2006) Proteomic identification of 14-3-3zeta as an adapter for IGF-1 and Akt/GSK-3beta signaling and survival of renal mesangial cells. Int J Biol Sci 3: 27-39.

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