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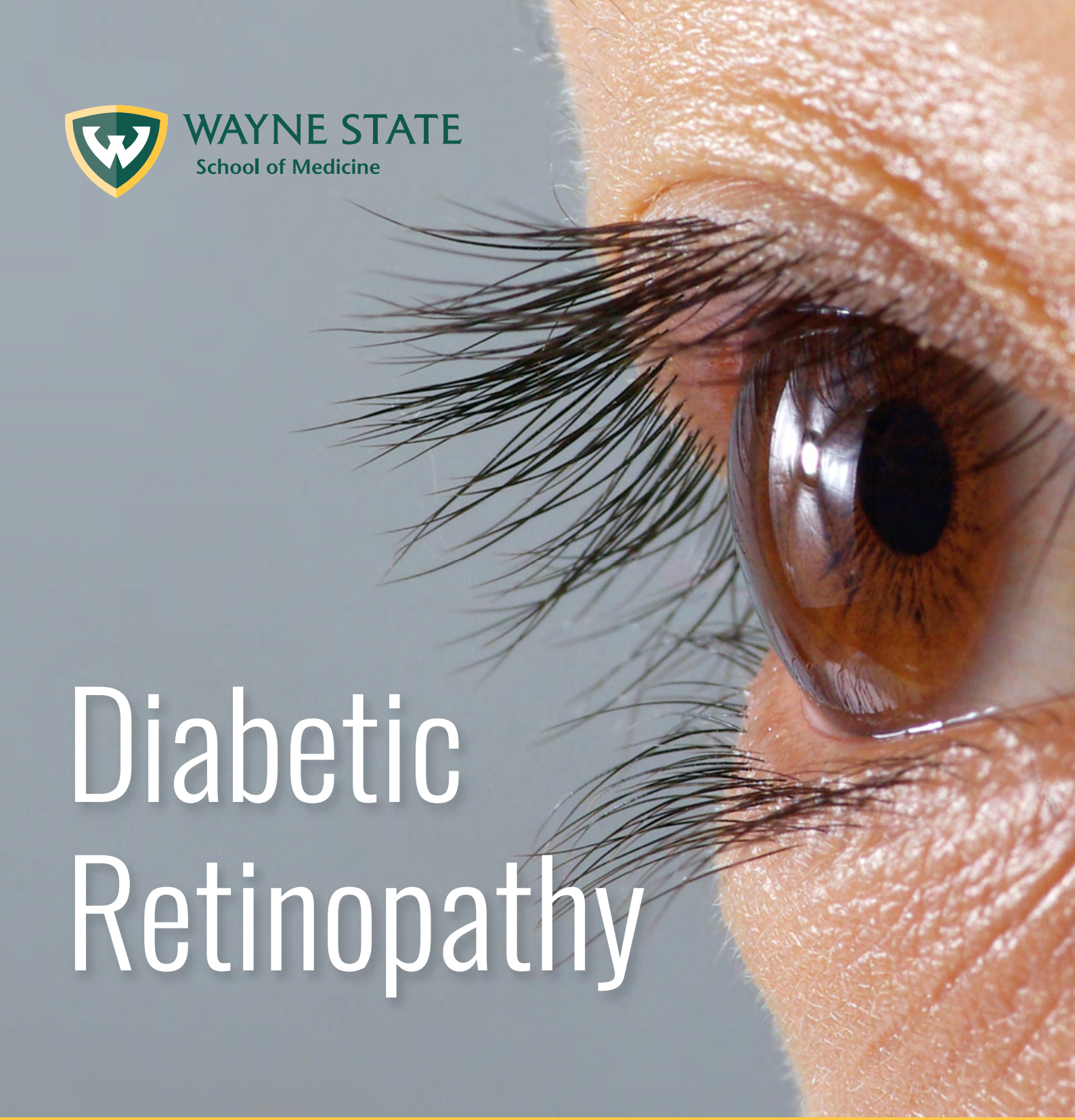
School of Medicine



Diabetic Retinopathy

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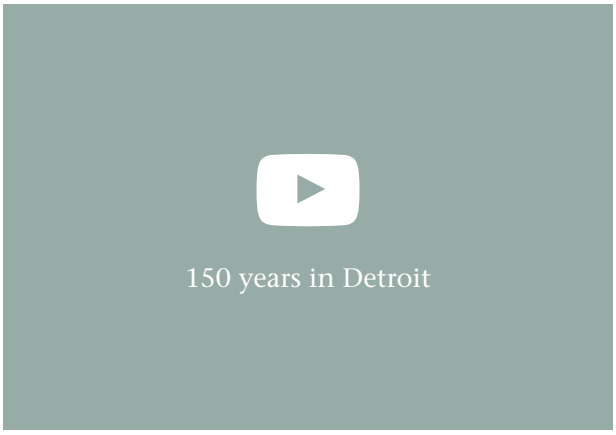
Thioredoxin-interacting protein (TXNIP) and pathogenesis of diabetic retinopathy

Dr Lalit Pukhrambam at Wayne State University School of Medicine is conducting research to find a cure for the blinding eye disease of diabetic retinopathy by targeting a protein called thioredoxin-interacting protein (TXNIP), as we find out here

Diabetic retinopathy (DR) is one of several organ complications of diabetes mellitus – a progressive microvascular disease leading to blindness. The number of patients with diabetes and DR will increase enormously in the coming years, and the familial and societal economic burden is enormous. Yet, there is no cure or preventive measures to tackle this devastating eye disease.

Whether it is Type 1 diabetes (T1D) or Type 2 diabetes (T2D) due to insulin deficiency or resistance, respectively, there is prolonged elevated glucose level in the blood and tissue causing cellular metabolic derailment. Therefore, hyperglycemia-induced cellular oxidative stress and chronic low-grade inflammation are considered to play critical roles in the etiology of DR. Most T1D and T2D diabetics will develop some forms of retinal complication within 10 to 15 years of diabetes duration. Early DR is manifested by retinal microvascular (blood vessel) aneurism, capillary leakage and exudes, which is described as non-proliferative DR (NPDR). NPDR then progresses to a more severe late histopathology of diabetes, which includes abnormal and weaker blood vessel growth (neovascularization), known as proliferative DR (PDR) leading to blindness. Also, blood vessel leakage at the centre of the retina (fovea) causes diabetic macular edema (DME) and visual distortion. Nonetheless, the cellular and molecular basis of DR pathogenesis remain elusive so far.

Current treatment methods include laser coagulation and anti-VEGF antibody injection in the vitreous; however, they are not curative and most patients do not respond to these treatments. Therefore, there is an urgent and unmet need for the identification of new pathogenic gene(s) and an understanding of the molecular and biochemical pathways in DR. Furthermore, in addition to early microvascular pathology (NPDR), currently, it is recognised that there is an early retinal neuronal injury and reduced visual function (e.g., contrast sensitivity) in diabetic patients before establishing retinopathy. This area of research is yet to be fully investigated and is not understood at present. Our laboratory at the Wayne State University School of Medicine is one group that is actively working to identify new targets and innovative therapies.



Wayne State University School of Medicine, Detroit

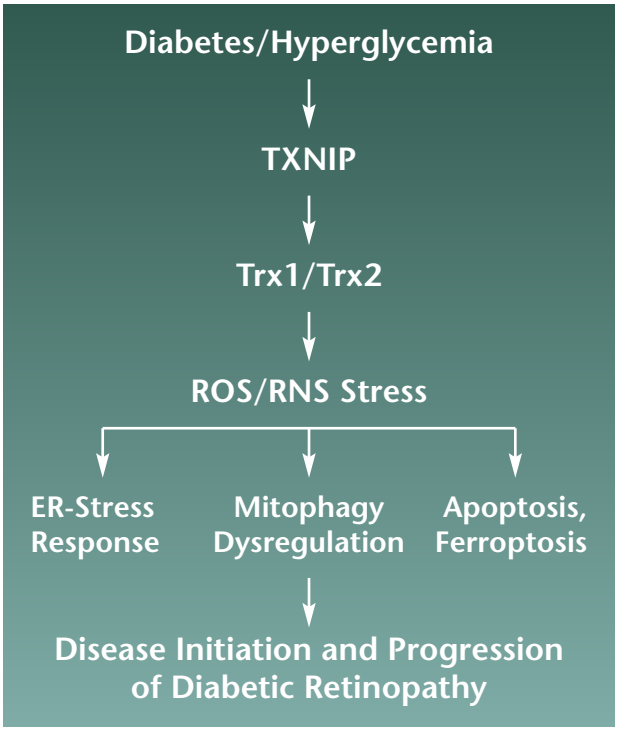
Thioredoxin-interacting protein (TXNIP)

One of the genes that are strongly induced by diabetes and high glucose in tissues including pancreatic beta, renal and retinal cells is the thioredoxin-interacting protein. TXNIP, as indicated by its nomenclature, binds to and inhibits the anti-oxidant and thiol-reducing capacity of thioredoxin (Trx) at its redox active site (32CXXC35) leading to cellular oxidative stress and inflammation. Trx1 isoform is present in the cytosol and nucleus while Trx2 is in the mitochondrion. TXNIP is localised in all these organelles and the plasma membrane. Therefore, TXNIP has been defined as pro-oxidative stress, pro-inflammatory and pro-apoptotic protein that is strongly activated in diabetes and its complications including DR.

The retina being a part of the central nervous system is made up of fully differentiated cells, therefore, they consume large amounts of glucose and oxygen for its bioenergetics and visual function. The mitochondrion inside the cell is the site of cellular energy production and involves in metabolism, innate immune response, and cell dead/live decisions. In addition, TXNIP is also involved in endoplasmic reticular stress response (ER-UPR) in various neurodegenerative diseases, where misfolded proteins accumulate causing irreversible protein repair and accumulation, assembly of the multiprotein complex NOD-like NLRP3 inflammasome and apoptosis.

TXNIP in mitophagy-lysosome axis dysregulation

As mentioned above, mitochondria are powerhouses of the cell, producing ATP as an energy source for the various cellular functions via its mitochondrial inner membrane electron transport chain (ETC) consuming molecular oxygen. Glucose metabolism by cytosolic glycolysis and mitochondrial tricarboxylic acid cycle (TCA or Kreb's cycle) produce electron donors (NADH and FADH2) for ATP production. During the process of the electron transfer in the ETC, some of the electrons leak out in the mitochondrial matrix and intermembrane space, where they are captured by molecular oxygen generating reactive oxygen species (ROS), which damage mitochondrial membrane lipids, proteins and DNA. Although there are anti-oxidant



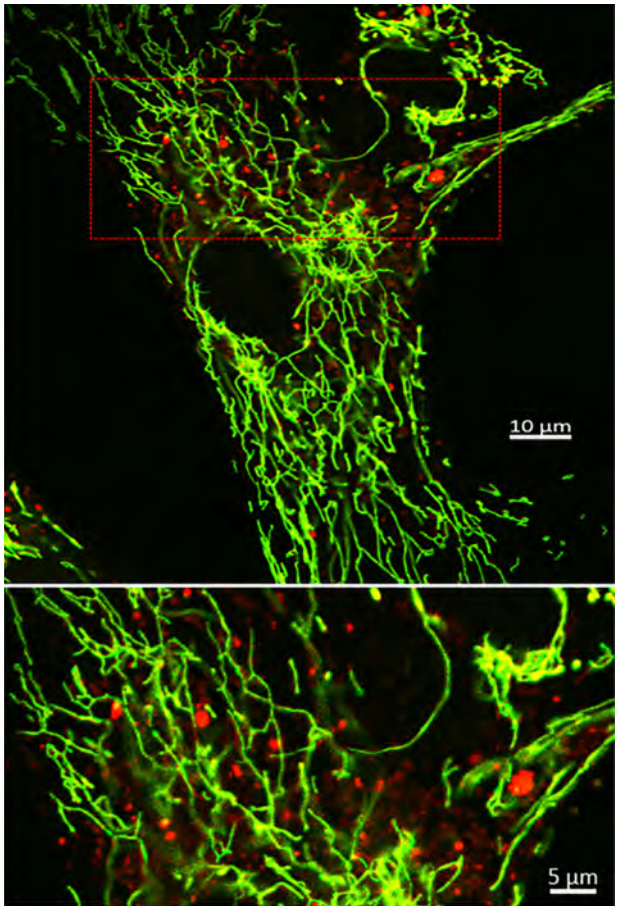
systems in the mitochondrion and cytosol to counter these harmful oxidants, however in disease conditions including DR, they are overwhelmed by the sustained production of oxidants including TXNIP inhibition of Trx1/Trx2.

Damaged mitochondria produce little ATP but continue to generate ROS. Furthermore, the mitochondrion, being a symbiotic protobacterium inside the cell, if released its constituents into the cytosol, they are recognised by cytosolic innate immune receptors (TLRs) as damage-associated molecular pattern (mtDAMP) and induce the assembly of the NLRP3 inflammasome, which activates caspase-1 and processes pro-inflammatory IL-1beta and IL-18 into their active forms inducing tissue inflammation. TXNIP itself involves the expression of pro-IL-1beta and assembly of the NLRP3 inflammasome and the processing of pro-IL-1beta under oxidative stress, resulting in inflammatory cell death by apoptosis or pyroptosis. Therefore, a coordinated removal of the damaged mitochondria and biogenesis of new ones are critical to prevent cellular stress while keeping an optimal mitochondrial number for efficient bioenergetics and metabolism.

An important aspect of TXNIP biology and function is its role in mitophagy, which is an autophagic process of removing damaged mitochondria via lysosomal degradation. Therefore, the segregation of damaged parts of the mitochondrion by fission, which involves dynamin-related protein 1 (DRP1, a GTPase) and fission protein fis1, and PINK1-Parkin-mediated mitophagic flux using mitophagy adaptors optineurin, p62 and LC3BII double-membrane autophagophore are essential. Furthermore, TXNIP interacts with REDD1 (regulated in development and DNA damage 1) and inactivates ATG4B, which leads to enhanced LC3BII formation. Dysregulation of autophagy and mitophagy leads to the accumulation of oxidized proteins and damaged mitochondria causing premature cell death. However, mitophagy is a double-edged sword – too much of it causes excess removal of mitochondria via mitophagic flux and lysosomal enlargement leading to a reduced number of mitochondria, bioenergetics deficiency/failure, and lysosomal membrane permeabilization (LMP) and leakage of acid hydrolases into the cytosol. Conversely, a slow mitophagy leads to accumulation of nonfunctional damaged mitochondria, excess ROS generation, and release of mtDAMP to the cytosol. Therefore, an optimal mitophagic flux is critical for the mitochondria-lysosomal axis homeostasis and cell survival.

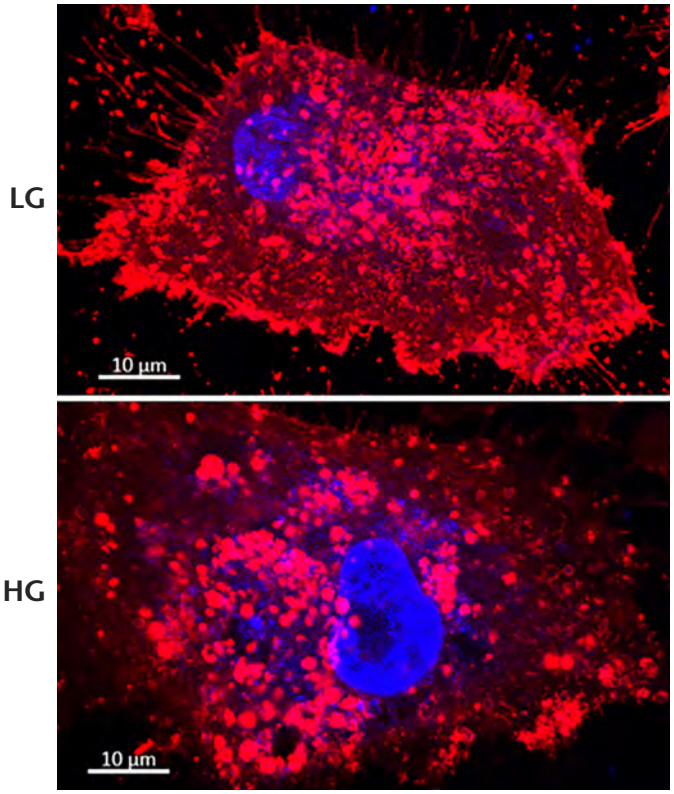
Nonetheless, physiological or pathologically optimal mitophagic flux rates are unknown and they may be different in different retinal cell types depending on their metabolic rates and bioenergetics requirement as well as on stages of the disease progression. Therefore, measuring an accurate and efficient mitophagic flux is necessary.

Human Retinal Pigment Epithelium, HRPE



Mt-Keima as a mitophagy probe

Human Retinal Pigment Epithelium, HRPE



LAMP1-mCherry as a lysosomal probe

Mt-Keima as a mitophagic probe:

Recently, mt-Keima was introduced to measure mitophatic flux in living cells and animal tissues using confocal microscopy. Mt-Keima is a coral protein, which when expressed in the mitochondrion emits a green light at alkaline or neutral pH (>7.0 pH). However, mt-Keima emits a red light after mitophagic flux to lysosomes, where an acidic pH (<5.0 pH) is encountered. Therefore, a single probe can monitor the mitophagic flux during normal physiology and under stress. We also have used this probe in our recent studies in retinal pigment epithelium under diabetic conditions. Therefore, a single probe, mt-Keima, can monitor mitochondrial filament network, fragmentation, and mitophagic flux to lysosomes.

LAMP1-mCherry as a lysosomal probe:

Another molecular probe we used to monitor lysosomal size and distribution is the LAMP1-mCherry. LAMP1 (Lysosome-associated membrane protein 1), is a membrane glycoprotein which together with LAMP2 makes up about 50% of the lysosomal membrane proteins. These proteins are involved in membrane recycling and repairing as well. We have observed that under sustained hyperglycemia (25 mM glucose, HG) in retinal pigment epithelial cells in culture, mitophagosome and lysosome fusion increases causing lysosomal enlargement and membrane destabilization and permeabilization, LMP. Such events cause leakage of lysosomal acidic hydrolases (such as Cathepsin L) into the cytosol and plasma membrane. Similarly, leakage of lysosomal cathepsin B may also cause cleavage of


Lalit Singh Pukhrambam

mitochondrial membrane proteins and mitochondrial damage. On the other hand, lysosomal fission and synthesis of new lysosomes may be blunted as there are a smaller number of lysosomes and their distribution appears to concentrate centrally, not towards the plasma membrane, which is readily seen under physiological glucose condition (5.5 mM, LG).

Transcription factor EB (TFEB) and CLEAR Network:

Upon mitophagy flux and lysosomal fusion, the lysosomal membrane protein mucolipin 1 or transient receptor potential mucolipin 1 (TRPML1), which is a lysosomal calcium channel, transiently opens to release local calcium and activates calcineurin phosphatase. One of the lysosomal membranes bound transcription factors is TFEB, which is phosphorylated by mTORC1, an important regulator of autophagy/mitophagy, trapping it in the cytosol by 14-3-3 scaffolds. Upon dephosphorylation by calcinuerin, TFEB is released, translocated to the nucleus where it activates the expression of a group of genes involved in lysosomal and autophagic function known as the CLEAR gene network. CLEAR denotes co-ordinated lysosome expression and regulated. In addition, TFEB and PPAR gamma cofactor PGC1alpha also activate each other to increase mitochondrial biogenesis to maintain the mitochondrial number and bioenergetics. Therefore, lysosome becomes a hub of autophagy, mitophagy and exocytosis of digested cargo materials as nutrients under starvation. However, under hyperglycemia and in DR, the mitophagy-lysosomal pathway may be disturbed leading to accumulation of undigested protein aggregates and oxidized mitochondrial lipoproteins as lipofucsin, which traps lysosomal acidic hydrolases leading to reduced enzyme activity. Therefore, the activation of TRPML1 using pharmacological agents and/or of TFEB may improve cellular function and prevent or delay the progression of DR. Upstream of TRPML1 activation is PIKFYVE, a kinase for PI3,5 bisphosphate, involved in endosomal and lysosomal trafficking.

In conclusion, TXNIP is strongly induced by diabetes and high glucose in diabetes and its complications, including DR. The TXNIP promoter exists in an open and poised configuration such that it is induced by diabetes and high glucose immediately and significantly. TXNIP remains upregulated as long as hyperglycemia prevails. Therefore, TXNIP causes cellular oxidative stress, dysregulation of the mitophagy-lysosomal axis and premature cell death. In addition, mitochondria are a major site of iron metabolism, required for TCA cycle and ETC assembly including iron-sulfur complexes and heme biosynthesis. Therefore, excess overloading of damaged mitochondria to lysosomes via mitophagic flux may cause lysosome membrane lipid peroxidation by Fenton reaction and cell death by ferroptosis, a recently identified iron-dependent cell death pathway due to lipid peroxidation and inactivation of antioxidant glutathione peroxidase 4 (GPX4). Such a process needs further exploration in

DR. In additions, gene and drug therapies targeting TXNIP and strengthening the mitochondria-lysosome axis may prove to be effective methods in treating neurovascular defects in DR. Current innovative research in DR in the US and around the globe is promising to find a cure for this blinding eye disease.

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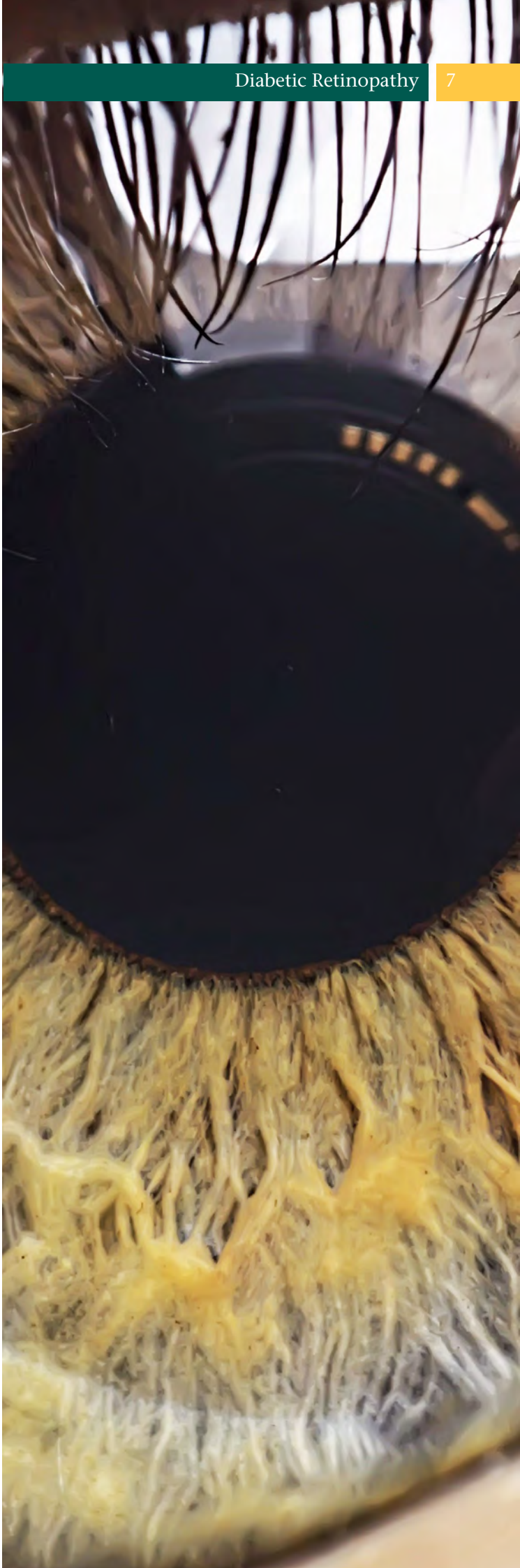

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