Abstract. Diabetic retinopathy (DR) is a frequent microvascular complication of advanced-stage diabetes. Endothelial cell dysfunction (ED) induced by diabetes plays an important role in the development of DR. It is considered that inflammation and mitochondrial homeostasis are associated with the progression of ED. Takeda G protein-coupled receptor 5 (TGR5) is a membrane receptor for bile acids (BAs) that plays an important role in regulating BA metabolism. Recent studies have shown that TGR5 is involved in regulating various mediators of ED and improving the dysfunction of vascular endothelial cells in DR; however, the exploration of specific related mechanisms remains an active research area in this field, which suggests that TGR5 may be one of the potential targets for the treatment of associated ED in DR. In the present review, the association between TGR5 and mitochondrial homeostasis was investigated. The extent of inflammation in DR-induced ED was assessed to provide possible evidence for the development of targeted therapies against DR.

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1. Introduction

Diabetic retinopathy (DR) is a common microvascular complication that presents at the late stages of diabetes. Approximately 80% of diabetic patients experience DR 20 years following onset, and its incidence is increasing worldwide (1,2). It has become one of the most important causes of blindness and visual impairment in working-age individuals (3,4). The initial stage of DR does not present with apparent symptoms; however, as the disease progresses, patients may experience blurred vision or even blindness (5,6). Early lesions in DR are characterized by loss of retinal capillary pericytes, resulting in increased vascular permeability and vascular leakage (7). Progression of DR to its later stage is followed by neovascular proliferation, which significantly increases the likelihood of visual loss (8,9). Endothelial cell dysfunction (ED) is the key element to the development of microvascular lesions. Certain studies have shown that hyperglycemia-induced oxidative stress is increasing, which stimulates the inflammatory pathways and promotes vascular dysfunction of the retina leading to increased capillary permeability and vascular leakage (10). In addition, mitochondrial homeostasis is associated with ED (11). The enzyme, endothelial nitric oxide synthase (eNOS), also plays a vital role in maintaining the function of endothelial cells (ECs) (12).

Bile acids (BAs) are a class of endogenous molecules synthesized in the liver; they are present in the bile as ionic salts derived from the metabolism of cholesterol (13). The main role of cholesterol is to promote the digestion and absorption of lipids. In the case of diabetes mellitus (DM), lithocholic acids and deoxycholic acids, formed by the enterohepatic cycle of BAs, have a high affinity for Takeda G protein-coupled receptor 5 (TGR5); the BA-induced activation of TGR5 increases glucagon-like peptide-1 (GLP-1) and insulin release (14). In recent years, a high number of studies...
have shown that BAs can be used as a signaling molecule to bind to the corresponding receptors and participate in the regulation of various metabolic diseases (15,16). TGR5 is a widely studied signaling molecule involved in this process. TGR5 is expressed in a variety of tissues and organs, such as the liver, kidney, brain, and heart (17,18). It is also widely expressed in almost all types of ECs and is involved in the regulation of glucose and lipid metabolism processes present in various metabolic diseases, such as obesity, non-alcoholic fatty liver disease, and type 2 diabetes mellitus (T2DM) (19,20).

Currently, farnesoid X receptor (FXR) is mainly reported to be related to the function of macrovessels, while TGR5 is less relevant (21). In addition, as a small tissue in the eyeball, the blood vessels distributed on the retina are mainly microvessels. From an etiological point of view, the specific pathogenesis of DR remains unelucidated, but the current view accepted widely by researchers is that DR is a retinal microvascular complication induced by the long-term hyperglycemic environment. Therefore, in the present review, the role TGR5 plays in microvessels was investigated and an attempt was made to elucidate the underlying possible mechanisms.

To date, various studies on BAs and their receptors have implicated their possible roles in the regulation of EC function (22). Previously, it has been revealed that TGR5 is highly expressed in retinal microvascular ECs (23), which may produce BAs through the ‘alternative’ pathway (24). It has also been found that intermittent fasting increases the production of taurodeoxycholic acid (TUDCA), a metabolite of BAs in the retina, and protects retinal ECs to delay the progression of DR (23). A previous study has shown that TGR5 agonists are beneficial in diabetes and TGR5 has become a promising target for the treatment of this disease (25). Therefore, it was hypothesized that TGR5 activation may delay the progression of DR by improving EC, which plays a protective role in the retina; however, the underlying mechanism remains to be elucidated in further studies.

In the present review, the role of TGR5 in delaying the progression of DR was summarized by its effect on maintaining mitochondrial homeostasis and counteracting inflammation to protect ECs from damage. Therefore, the present study aimed to provide possible evidence for the application of the targeted therapy of DR.

2. DR and ED

ECs are a layer of squamous epithelial cells covering the inner surface of blood vessels, which constitute a barrier between blood vessels and tissues and control the transport of substances between tissues and blood vessels. ECs act as a metabolic interface between the blood and the tissues and are important in maintaining the stability of the intravascular environment (26). ED occurs when ECs are unable to maintain homeostasis of the vascular environment. It is a systemic pathological condition characterized by changes in the phenotype of ECs, which leads to diminished vasoconstriction and the formation of a proinflammatory and prothrombotic state (27). ED forms the basis of the chronic microvascular and macrovascular complications of diabetes. In recent years, significant progress has been made in understanding the mechanism of ED and its pathogenesis in patients with type 1 diabetes mellitus (T1DM) and T2DM. Several factors that cause ED have been identified and the common causes include hypoxia, aging, hyperglycemia, hypercholesterolemia, and hypertension (28). Previous studies have shown that pathological processes caused by a high glucose environment found in diabetics, such as inflammation, oxidative stress, and endoplasmic reticulum (ER) stress are responsible for the continuous progression and aggravation of ED in the course of the disease (29). As a common microvascular complication in the late stage of diabetes, the risk factors for the development of DR are mainly related to the severity and exposure time of hyperglycemia, hypertension, and hyperlipidemia (30). ED is the pathological basis of diabetic microvascular complications and plays an important role in the pathological progression of DR. Progressive dysfunction of ECs will certainly lead to changes in morphological structures, such as capillary basement membrane thickening, perivascular cell loss, BRB damage, and neovascularization, which accelerates the progression of DR (31,32).

3. DR and mitochondrial homeostasis and inflammation

The specific mechanisms leading to DR have not been fully elucidated. However, disruption of mitochondrial homeostasis and inflammation are considered to be closely related to the pathogenesis of DR (33,34).

Diabetes can disturb mitochondrial dynamic homeostasis, causing impaired mitochondrial function, which in turn causes the development of related diseases (35,36). Under high glucose conditions, the electron flux through the electron transport chain increases, eventually leading to increased reactive oxygen species (ROS) production, which in turn causes retinal damage (37,38). The mitochondrial fusion division mechanisms are also compromised in diabetes; swollen retinal mitochondria decrease mitofusin 2 (Mfn2) expression and increase dynamin-related protein 1 (Drp1) expression (39). Decreased mitosis and inflammation activation can be observed in the retina of diabetic patients (40), which further leads to deterioration of mitochondrial homeostasis.

In addition, several studies have implicated various systemic and local inflammatory factors in DR (41,42). Diabetes causes increased local and systemic expression of inflammatory cytokines, chemokines, and growth factors, all of which are involved in the development of DR (43-45). It has been shown that fortified extracts of red berries, ginkgo biloba leaves, and white willow bark containing carnosine and α-lipoic acid can significantly reduce cytokine levels in the retina and inhibit lipid peroxidation, which is associated with diabetes (46). Another study has demonstrated that curcumin can protect against high glucose-mediated retinal pigment epithelial cell injury due to induction of an anti-inflammatory pathway (47). Purinergic signaling has been shown to be a key factor in regulating the inflammatory status in different organ tissues. P2X purinergic receptor 7 (P2RX7) is a common purinergic ionotropic receptor; its activation leads to the release of proinflammatory mediators and the induction of cell damage. This receptor is considered to be a target for restoring BRB and reducing inflammation. It has been experimentally demonstrated that the inhibition or downregulation of P2X7R
expression plays a protective role in inflammation-induced cell damage (48-50).

4. Structure of TGR5

TGR5, also known as G protein-coupled BA receptor 1 (GPBAR1) (51), belongs to the class A G-protein coupled receptor (GPCR) subfamily. The receptor comprises seven transmembrane helices (TMH1-7), three extracellular loops (ECL1-3), contributing to ligand binding, and three intracellular loops (ICL1-3) involved in mediating the signal to downstream signaling molecules (52). Substantial evidence has demonstrated that purinergic receptor P2Y9 in position 3.33 and chemokine receptor CXC3 in position 5.43 is involved in key ligand interactions. In addition, the receptor around Ser270 can recognize the acidic side of BAs and the hydrophobic pocket (including Phe96, Cys236, and Trp237) in TGR5 can host the C6 and C7 positions of BAs. The intracellular terminal of TM6 on TGR5 and GαS-Ras domain can interact with each other and activate the second messenger. TGR5, Takeda G protein-coupled receptor 5; BAs, bile acids.

5. TGR5 and ED

TGR5 is a common membrane receptor during BA metabolism and has been demonstrated to be expressed in a variety of tissues and organs (54). The role of TGR5 in regulating homeostatic metabolism is also well documented. A previous study has shown that TGR5 can delay the occurrence and development of portal hypertension by reversing ED (56). It was also found that activation of TGR5 could reverse the injury of liver sinusoidal ECs in a mouse model of cirrhosis and could reverse cardiovascular injury by reducing the secretion of inflammatory factors in aortic intimal cells (56,57). These studies indicate that activation of TGR5 may be a potential therapeutic strategy to delay ED caused by DR.

6. TGR5 and mitochondrial homeostasis

Previous studies have confirmed a close association between the damage of ECs and mitochondrial damage. Under damaged conditions, mitochondria generate large amounts
of ROS, such as superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), peroxyl radical ($ROO^·$), and reactive hydroxyl radical ($·OH$), which are generally considered harmful to cells (58,59). Concomitantly, oxidative stress can cause changes in mitochondrial dynamics, such as activation of mitochondrial fission, inhibition of mitochondrial fusion, and an increase in the levels of mitophagy (60,61). Mitochondrial fusion involves three proteins, namely Mfn1, Mfn2, and optic atrophy 1 (OPA1), while mitochondrial fission is mediated by Drp1 and its receptors, including mitochondrial fission factor (MFF) and fission 1 (Fis1) (62,63). High levels of ROS can generate the release of inflammatory factors, such as vascular endothelial growth factor (VEGF), IL-1β, and TNF-α. For example, TNF-α can cause leakage of blood vessels by upregulating NK-κβ to activate ICAM-1 (64) and induce EC damage. Numerous experiments have demonstrated that the production of a series of inflammatory cytokines and the changes in mitochondrial dynamics in diabetic rats contribute to retinal microvascular endothelial cell (RMEC) dysfunction in this animal model (65,66) (Fig. 2).

Mitochondria are the main site of energy production and play a crucial role in energy conversion and metabolism. In addition, mitochondria perform various functions that are essential for cell survival and have to maintain these processes and also adapt to the changing cellular environment. Mitochondria, as highly mobile double-membrane organelles, can form dynamic and extensive cellular networks that maintain homeostasis through fusion, fission, and mitophagy (62). Normally, fusion and division of mitochondria exist in a dynamic equilibrium. Mitochondrial dynamics are essential for the regulation of mitochondrial function and mitochondrial fragmentation has been shown to be involved in the induction of pathological processes including DM (67-69).

Substantial evidence has demonstrated that therapies that improve mitochondrial function can ameliorate damage to retinal ECs. D-Arg-dimethylTyr-Lys-Phe-NH2 (SS-31) is a mitochondria-targeted antioxidant peptide, which effectively reverses the decreased visual acuity in a streptozotocin-induced diabetic mouse model (70). Huang et al (71) demonstrated that diabetic rats treated with SS-31 exhibited improved retinal ganglion cell structure, thinner capillary basement membrane, and reduced inner BRB leakage. Therefore, the improvement of mitochondrial damage may become a new strategy to treat DR.

ECs rely on glycolysis for energy supply, which may be a misleading concept suggesting that adenosine triphosphate (ATP) derived from mitochondria has no important role in ECs (72). However, recent evidence suggests that while the energy requirements between ECs are not as large as those of cardiomyocytes and smooth muscle cells, intracellular ATP may play an important role in mediating the normal physiological functions of ECs (73). Mitochondrial oxidative phosphorylation plays an integral role in energy stores...
and mitochondrial dysfunction can enhance oxidative stress sensitivity and lead to EC death (65). Therefore, a decreased mitochondrial function also contributes to ED. Recently, it has been shown that EC injury can be delayed by reducing mitochondrial division and/or enhancing mitophagy via the activation of TGR5.

It has been shown that INT-777, an agonist of TGR5, prevents mitochondrial division by decreasing calcium concentration, attenuating protein kinase C (PKC) activation, and inhibiting the Ca^{2+}-PKCδ/Drp1 pathway (65). It has also been found that PKCδ can lead to Drp1 phosphorylation and translocation of phosphorylated Drp1 to mitochondria can promote mitochondrial division (74). Intracellular calcium can activate calcineurin to promote mitochondrial division and induce activation of Ca^{2+}/calmodulin-dependent protein kinase II, which can mediate p-S616 expression in Drp1 (75,76). Activation of TGR5 can reduce intracellular Ca^{2+} concentrations by blocking the influx of extracellular Ca^{2+}, thereby inhibiting mitochondrial division. Concomitantly, decreased intracellular Ca^{2+} inhibits the extracellular regulated protein kinases (ERK1/2) signaling pathway, which can cause a decrease in Drp1 expression; this, in turn inhibits mitochondrial division and promotes Mfn1 oligomer formation, thereby promoting mitochondrial fusion (77-79).

Physiologically, a low number of damaged mitochondria are formed during mitochondrial fission, and damaged mitochondria are degraded by mitochondria-targeted autophagy termed mitophagy (80). TGR5 can activate the PTEN-induced kinase (PINK)/Parkin pathway and inhibit the PKCδ/Drp1-hexokinase (HK)2 pathway to enhance mitophagy. HK is a positive modulator of Parkin recruitment and glycolysis (81,82). As the major HK isoform in insulin-sensitive tissues including retinopathy, HK2 binds to voltage-dependent anion channels and localizes to the outer mitochondrial membrane. HK2 translocates from the mitochondria into the cytosol in response to high glucose conditions in diabetic mice. Treatment with INT-777 promotes recruitment of HK2 to the mitochondria and further activation of the PINK1/Parkin signaling pathway. The use of the Drp1 inhibitor Mdivi-1 can promote, in a similar manner, the translocation of HK2 from the cytosol to the mitochondria. This suggests a role for TGR5 in enhancing mitophagy and inhibiting cell division in vitro. In vivo, capillary degeneration and pericyte loss has been shown to be milder in TGR5-knockdown rats injected with the mitochondrial fission inhibitor Mdivi-1 or the mitophagy agonist rapamycin compared with that noted in control animals (65). In summary, TGR5 maintains mitochondrial homeostasis by reducing mitochondrial division and enhancing mitophagy, which in turn improves ED to delay the progression of DR (Fig. 3).

7. TGR5 and inflammation

DR is classified as a chronic low-level inflammatory process and accumulating evidence has shown that minor
inflammation is responsible for the vasculopathy of DR. In the presence of oxidative stress caused by hyperglycemia, the levels of inflammatory factors, such as cytokines, VEGF, IL-1β, and TNF-α, in the serum and local microenvironment are increased. This increase in inflammatory factors leads to retinopathy (83). Previous studies have demonstrated that activation of the TGR5 receptor can delay the production of IL-1, TNF-α, and other inflammatory factors by macrophages; it may also reduce the production of proinflammatory factors by inhibiting the Toll-like receptor (TLR)4/NK-κβ pathway and can play a role in inhibiting the phosphorylation of STAT3 (21,84,85). Among all inflammatory factors, TNF-α was first implicated in the progression of insulin resistance, as well as in abnormal glucose metabolism associated with T2DM. Therefore, the association between inflammation and DR was assessed with regard to the contribution of TNF-α.

It has been reported that TNF-α can activate various caspases leading to apoptosis of inflammatory cells in the chronic inflammatory response. TNF-α can also activate NK-κβ, thereby causing an upregulation in the expression levels of related genes involved in inflammation and resulting in increased intercellular adhesion molecule 1 (ICAM-1) synthesis. A large amount of ICAM-1 will damage vascular ECs following binding to activated leukocytes, resulting in vascular leakage at the corresponding site (64). Several lines of evidence suggest that the elevation of TNF-α is significantly associated with diabetic angiopathy in diabetic complications. The experiments indicated higher TNF-α levels in the serum of diabetic rats than those noted in normal mice in vivo. Following treatment with apigenin and ramipril, the increase in the levels of TNF-α was inhibited. Moreover, glomerular hypertrophy, fibrosis, and matrix expansion were improved, and the degree of inflammation was reduced in diabetic rats (86). In vitro, it has been demonstrated that the mRNA expression and secretion of TNF-α are markedly upregulated in human glomerular EC cells (HRGECs) treated with high concentrations of glucose (87). A recent study has also demonstrated that TNF-α levels are elevated in the early stages of retinopathy and remain high throughout the process; therefore, it is speculated that TNF-α can be used as a marker to predict DR (88).

TGR5 has been found to regulate TNF-α by modulating the Rho/Rho-associated protein kinase (ROCK) signaling pathway. As an agonist of TGR5, INT-777 can block TNF-α-induced RMEC proliferation and migration and inhibit the effect of TNF-α on promoting vascular permeability (66,89). In addition, previous evidence has shown that TGR5 agonists can upregulate IL-10 expression to exert anti-inflammatory and immunosuppressive effects and reduce the expression of the proinflammatory cytokines IL-1β, IL-6, and TNF-α by activating the TGR5-cAMP-protein kinase A (PKA) signaling pathway (90,91). In addition to the two classical signaling pathways described above, GLP-1, an intestinal hormone with
a short half-life, has been shown to inhibit inflammation and improve EC function (92). A study has shown that GLP-1 can delay the damage of ECs by inhibiting NF-κB, which in turn inhibits the secretion of inflammatory factors, such as IL-1β, IL-6, and TNF-α (93). While various studies confirm that GLP-1 is one of the targets of TGR5; its secretion is dependent on TGR5 (93,94). Therefore, it was speculated that activation of TGR5 may be an effective way to stimulate GLP-1 secretion and delay RMEC injury (Fig. 4).

8. Discussion and future perspectives

As previously mentioned, oxidative stress, inflammation, and mitochondrial damage are important mechanisms of DR that lead to EC damage. The activation of TGR5 can reduce TNF-α expression via the Rho/ROCK pathway as well as promote the secretion of GLP-1 (66,94). TGR5 affects microsomal kinetics by inhibiting the Ca<sup>2+</sup>-PKCδ/Drp1 pathway, upregulating the PINK/Parkin pathway, and regulating the PKCδ/Drp1-HK2 pathway to alter DR. Therefore, the ability of TGR5 to ameliorate EC function may be one of the potential mechanisms responsible for its inhibitory effect on DR.

In addition to the aforementioned mechanisms, activation of TGR5 stimulates vascular ECs to produce eNOS to maintain vascular health and function; this mechanism has also the potential to improve DR (95). Previous studies have shown that activation of TGR5 can effectively increase eNOS expression; and its expression level is increased through the bile salt-TGR5-cAMP pathway and the TGR5-GLP-1-eNOS pathway. (17,27). In addition, a study has also shown that tauroliothiocolic acids (TLCA), taurocholic acid (TCA), and taurochenodeoxycholic acid (TCDA) as agonists of eNOS has been shown to elevate eNOS expression and Ser1177 phosphorylation of this enzyme, leading to increased nitric oxide (NO) production (96). The increase in NO production can effectively protect the vascular endothelium.

Exchange proteins directly activated by cAMP (EPACs), consisting of Epac1 and Epac2, are cAMP mediators independent of PKA. As a mediator of cAMP, Epacs take part in numerous biological functions (97,98). Increasing studies in recent years have demonstrated the role cAMP/Epac signaling plays in endothelial cell barrier function (99,100). It has been found that Epac-1 expression is significantly reduced in mouse models of DR, suggesting that Epac-1 is a critical regulator of endothelial function in diabetic microangiopathy involving endothelial dysfunction associated with hypoxia. Activation of Epac-1 by forskolin or the cAMP analog 8-pCPT reduces its sensitivity to oxidative stress, restores the endothelial permeability barrier, rescues NO production by eNOS and inhibits ROS formation (101), suggesting that Epac-1 may be a potential target for the treatment of ED during DR. It has also been revealed that activation of Epac inhibits VEGF receptor signaling through the Ras/MEK/ERK pathway to improve BRB permeability (102). In addition, Epac has also been demonstrated to reduce inflammatory mediators in retinal endothelial cells, potentially mediating anti-inflammatory responses in endothelial cells (103). In the present review, it was indicated that TGR5 can activate cAMP, from which it can be speculated that TGR5, after activating cAMP, may improve retinal endothelial cell function through cAMP/Epac signaling, thereby delaying the progression of DR.

To date, insufficient evidence has been reported supporting the notion that TGR5 can delay vascular endothelial injury in DR through ER stress. Therefore, it is possible that a new mechanism may be responsible for this process. Achieving a relatively balanced state of mitochondria by maintaining the homeostasis of the ER has the potential to be a novel mechanism by which TGR5 delays EC injury. ER stress, such as interference in Ca<sup>2+</sup> homeostasis, redox imbalance, and defects in protein folding can cause disorders in ECs (104). In ER stress, the unfolded protein response (UPR) can be triggered and the UPR is an adaptive process to restore ER stress. It has been shown that oral administration of TUDCA can reduce ED triggered by hyperglycemia. Moreover, activation of the UPR by the use of the chemical chaperone TUDCA can alleviate the glucose-induced increase in inflammatory cytokines and endothelin-1, as well as the decrease in NO levels (105,106). It has been demonstrated that ER stress is associated with TGR5 and that TGR5 mRNA levels are upregulated in skeletal myotubes in response to the UPR inducers thapsigargin (ER-specific Ca-ATPase inhibitor) and tunicamycin (N-glycosylation inhibitor), demonstrating that TGR5 is a novel UPR target gene (107). Additional studies have demonstrated that TUDCA can reduce ER stress by stimulating the TGR5 signaling pathway (108,109). It has been shown that upregulation of TGR5 expression inhibits the TLR4/NF-κB pathway to reduce oxidative stress, which may delay endothelial injury (85). TGR5 may alleviate ER stress through the protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK)/eukaryotic initiation factor 2(α/eIF2)/NF-κB pathway and activating transcription factor 4-CCAAT-enhancer-binding protein homologous protein (ATF4-CHOP); therefore, these targets have the potential to be used for delaying necrosis of diabetic RMECs (104).

Since the activation of the TGR5 receptor improves EC injury caused by mitochondrial injury, oxidative stress, inflammatory factors, and ER stress, it may play a role in delaying DR-induced ED. Therefore, the TGR5 receptor can be used as a new target to ameliorate DR. Quinoa can cause an upregulation of the expression of GLP-1 via increased expression levels of TGR5 and it may be useful in the treatment of DR (85). Therefore, it is possible that certain components in Chenopodium album may act as agonists of TGR5. This application can be supported further by in vivo studies and clinical trials in humans.

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MZ conceived and designed the article. MZ and ZD prepared and wrote the manuscript. MZ, ZD and WD performed a literature search, selected the studies to be included and drew the figures. MZ, ZD, DZ and XR revised the manuscript. DZ retrieved and analyzed relevant documents. Data authentication is not applicable. All authors have read and approved the final manuscript.

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The authors declare that they have no competing interests.

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