Study on the Regulatory Mechanism of the Gs Protein Signaling Pathway in Diabetic Retinopathy

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Abstract: Diabetic retinopathy is one of the microvascular complication induced by primary diabetes, characterized by neurovascular unit damage and blood-retinal barrier (BRB) breakdown. It causes visual impairment of varying severity, ranging from mild vision loss to complete blindness. Although the Gs pathway is not the core pathway in DR pathogenesis, it contributes to DR progression and holds important research significance. The Gs protein pathway mainly regulates physiological processes including cell proliferation, differentiation, and homeostasis maintenance through the Gs-cAMP-PKA signaling cascade. In addition, the Gs pathway is essential for the regulation of inflammatory responses and oxidative stress. The present study is based on the rationale that a persistent hyperglycemic environment caused by primary diabetes suppresses the Gs pathway, leading to dysfunction of its downstream regulated processes and the occurrence of secondary disorders such as DR. By elaborating the pathogenesis of DR in detail, this review aims to identify current research gaps, clarify the potential value of this pathway in DR treatment and drug development, and promote the translation of basic research into clinical applications.

Keywords: Diabetic Retinopathy; Gs Protein pathway; Oxidative Stress; Inflammation; Regulatory Mechanism

1. Introduction

Diabetic retinopathy (DR) is one of the most common complications of diabetes with a high rate of blindness[1]. Clinically, intravitreal injection of anti-vascular endothelial growth factor (VEGF) agents is the mainstay treatment for DR. However, such therapy leads to tolerance and adverse reactions in some patients, and is only effective for moderate to advanced lesions with poor efficacy in early-stage DR. Therefore, novel core regulatory mechanisms urgently need to be identified[1,2]. DR is characterized by severe vascular abnormalities, blood-retinal barrier breakdown, and neuronal dysfunction induced by hyperglycemia, with inflammation, oxidative stress, and hyperglycemia as central pathological events[2]. G protein-coupled receptors are widely expressed membrane receptors that play crucial roles in cellular signal transduction. Among the numerous subtypes, the Gs-cAMP-PKA pathway, dominated by the Gs protein subtype, extensively regulates various physiological functions to maintain normal cellular activities and systemic homeostasis. The retina, as a key site affected in DR, is also regulated by the Gs pathway[3]. However, current studies on the mechanism by which the Gs pathway regulates DR progression are fragmented and lack systematic summary. The overall regulatory network and key functional nodes of the Gs pathway in DR have not been fully elucidated. This review systematically summarizes the regulatory mechanism ((e.g., Figure 1)and research progress of the Gs pathway in DR, sorts out existing gaps, and provides new ideas for early intervention and target development of DR. The framework of this paper is illustrated in Figure 1.

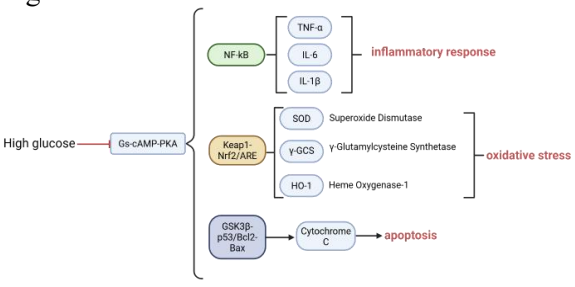

Figure 1. Research Ideas of This Review

2. Basic Regulatory Mechanism of the Gs Protein Pathway

2.1 Composition and Core Transduction of the Gs Protein Pathway

G protein-coupled receptors are monomeric

proteins that span the cell membrane seven times; they are also referred to as seven-transmembrane receptors and are widely distributed in organsim. G proteins are generally composed of α, β, and γ subunits, and are classified into four major types (Gs, Gi, Gq, and G12) based on the α subunit[3]. The core components of the Gs protein pathway include the Gαs subunit, adenylyl cyclase (AC), cyclic adenosine monophosphate (cAMP), and protein kinase A (PKA). Upon binding to certain ligands (hormones, peptides), GPCRs undergo conformational changes, leading to the dissociation of the Gα subunit from the Gβγ complex. The Gα subunit acts as a primary messenger to activate downstream adenylyl cyclase (AC), a key enzyme that catalyzes the conversion of ATP into cAMP. As a second messenger, cAMP further activates PKA, which regulates cellular functions by phosphorylating specific serine and threonine residues in target proteins[4]((e.g., Figure 2).

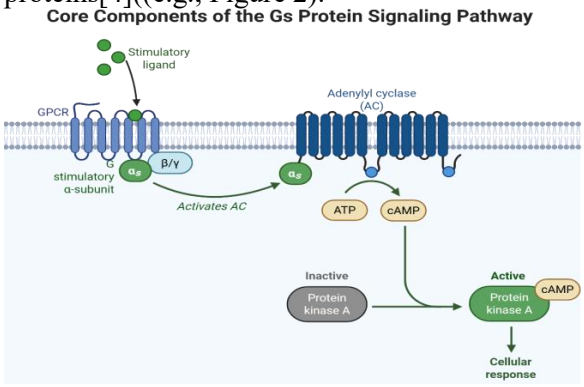

Figure 2. Core Components of the Gs Protein Signaling Pathway

2.2 Expression and Physiological Roles of the Gs Pathway in Retinal Tissue

GPCRs are widely expressed in retinal cells including Müller cells, microglia, and retinal pigment epithelial (RPE) cells, which are thus regulated by the Gs pathway[5]. The core mechanism involves the dissociation of the Gαs subunit to activate AC, catalyzing ATP into cAMP to increase intracellular cAMP levels, and subsequently activating PKA to regulate cellular metabolism and signal transduction, maintaining normal cellular homeostasis and metabolism. Microglia mainly exert immune functions in retinal tissue and a key glial cell population[6]. They are activated early in DR to release pro-inflammatory and neurotoxic mediators. Studies have shown that in diabetic rats, activated microglia exhibit enhanced proliferation, increased number, and elevated migration ability, moving from the inner to the outer retina and even into the subretinal space and RPE layer, leading to BRB breakdown, increased leukocyte leakage, and acellular capillary formation[6]. Müller cells release various inflammatory mediators and are a major source of VEGF, acting as a key initiator of inflammation in DR. Evidence indicates that growth factors, cytokines, chemokines, and other mediators present in the vitreous of diabetic patients are mainly derived from Müller cells[6].

3. Regulatory Roles of the Gs Protein Pathway in Core Pathological Processes of DR

3.1 Regulation of High Glucose-Induced Retinal Inflammation

Inflammation is indispensable in the pathogenesis of DR. Inflammation is a defensive response of the body against external stimuli; however, excessive inflammatory activation may further exacerbate tissue damage. In retinal tissue, Müller cells, monocyte-derived macrophages, and microglia are the major cell types mediating inflammatory responses, while inflammatory factors including IL-6, IL-1β, and TNF-α are the key molecules triggering inflammation. Studies have shown that levels of IL-6, IL-1β, and TNF-α are significantly elevated in the retina and vitreous fluid of DR patients.

Impaired function of the Gαs subunit is the core target of Gs pathway inhibition by high glucose. The Gαs subunit contains a highly conserved key site Arg201((e.g., Figure 3), a specific residue for ADP-ribosylation catalyzed by cholera toxin that directly controls the conformational activation of Gαs.

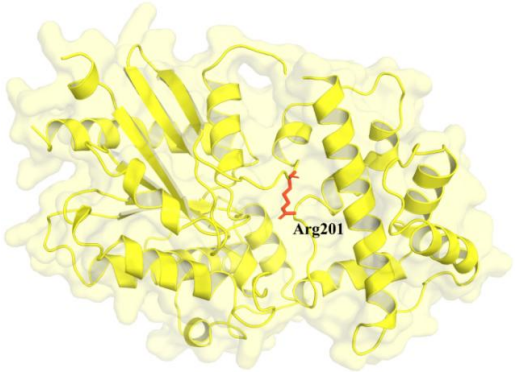

Figure 3. Structure and Key Regulatory Sites of Gsα Subunit

Under normal conditions, this site is exposed for proper activity regulation; however, high glucose drastically reduces the exposure of this ADP-ribosylation site, impairing Gαs activation and suppressing the Gs-cAMP-PKA pathway[7]. Related studies have shown that total Gαs protein remains unchanged in diabetic rats, but its ADP-ribosylation level is significantly reduced, indicating that high glucose inhibits Gαs through post-translational exposure of the site rather than expression level[7]. High glucose also inhibits adenylyl cyclase (AC). Glucose metabolism affects intracellular cAMP levels: elevated intracellular glucose enhances metabolism, increasing metabolites such as glucose-6-phosphate and UDP-GlcNAc, which inhibit AC and reduce cAMP production. High glucose also activates the PKC pathway, which antagonizes the PKA pathway and competitively inhibits AC activation. Additionally, abnormal cAMP-PKA signaling under high glucose is related to reduced Gαi protein expression, though the specific mechanism remains unclear[8].

Under physiological conditions, PKA inhibits NF-κB, preventing NF-κB and other transcription factors from regulating the transcriptional upregulation of key inflammatory cytokines including IL-6, IL-1β, and TNF-α, thereby alleviating cellular inflammation[9]((e.g., Figure 4). inhibitory effect of the cAMP-PKA signaling pathway on NF-κB activity is closely associated with the C-terminal transactivation domain of the p65 subunit. Further studies have demonstrated that this critical domain can undergo phosphorylation at multiple serine residues (Ser529 and Ser536) in response to various upstream stimuli.. PKA suppresses NF-κB by directly or indirectly modifying the phosphorylation of the p65 C-terminal transactivation domain. In high glucose conditions, Gs pathway inhibition reduces PKA expression and activity, relieving the repression of the p65 C-terminal transactivation domain and enhancing NF-κB transcriptional activity, thus upregulating NF-κB-dependent key pro-inflammatory cytokines (IL-6, TNF-α, IL-1β)[9]. Furthermore, PKA stabilizes IκB to promote its stable association with NF-κB, thereby inhibiting NF-κB transcriptional activity. In a high-glucose environment, decreased PKA activity leads to IκB destabilization and degradation, enabling enhanced downstream transcriptional activity of NF-κB[9].

Microglia, Müller cells, and other glial cells are regulated by the NF-κB pathway to maintain retinal homeostasis. Pathological imbalance of this pathway leads to massive release of inflammatory factors. IL-6 disrupts tight junctions between endothelial/epithelial cells, increasing RPE permeability and disrupting ZO-1 distribution to cause BRB breakdown[10]. It also activates the STAT3/VEGF pathway to downregulate ZO-1 and occludin, elevating vascular endothelial permeability and aggravating inflammation. IL-6 can further promote the release of TNF-α and VEGF[10].

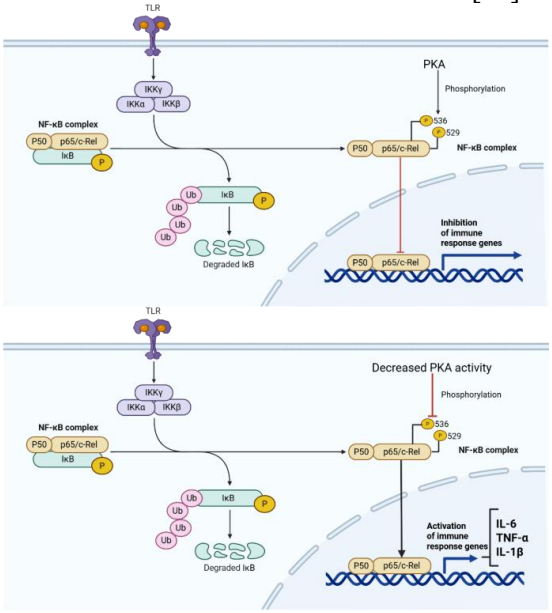

Figure 4. Regulatory Mechanism of the Gsα Pathway on NF-κB under High-Glucose Conditions (Upper: Normal Condition / Lower: High-Glucose Condition)

TNF-α plays a critical role in DR. Under high glucose, retinal Müller cells secrete TNF-α, activating certain signaling pathways (EGFR/p62) in RPE cells upregulates mitophagy and induces apoptosis. Studies have shown that TNF-α upregulates EGFR activity through phosphorylation and activates downstream p38. p38 can phosphorylate IKK, thereby promoting the degradation of IκBα and relieving its inhibitory effect on the NF-κB family. As a result, the transcriptional activity of NF-κB is enhanced, accompanied by increased expression of apoptosis-related genes. Cells release Bax/Bcl-2, cytochrome C, and cleaved caspase-3 to initiate the apoptotic program, trigger mitophagy, induce RPE cell apoptosis, and disrupt tight junctions between epithelial cells. As a key component of the blood-retinal

barrier, damage to RPE cells inevitably aggravates inflammatory responses and accelerates disease progression. Studies have confirmed that blocking the binding of TNF-α to EGFR alleviates blood-retinal barrier (BRB) breakdown in diabetic mice[11].

IL-1β is another key inflammatory factor affecting retinal barrier integrity[12]. In retinal tissue, IL-1β is mainly derived from Müller cells. Hyperglycemia activates the caspase-1/IL-1β pathway, initiating pyroptosis. Endothelial cell injury in DR is partially attributed to the long-term release of IL-1β from Müller cells. Increased release of certain inflammatory factors such as IL-6 and TNF-α is also related to IL-1β, mediating chronic inflammation and neovascularization. Studies have shown that inhibiting receptors associated with caspase-1 activation or IL-1β in retinal and vitreous tissues of diabetic patients can delay the progression of DR.[12].

3.2 Regulation of High Glucose-Induced Retinal Oxidative Stress and Apoptosis

Under physiological conditions, free radicals with strong oxidizing properties are continuously produced during metabolism, while the body synthesizes antioxidant proteins to scavenge free radicals, maintaining a balance between oxidation and antioxidation. External stimuli disrupt this homeostasis, causing oxidative stress and cellular damage. Oxidative stress is therefore crucial in DR progression.

Nrf2 is a transcriptional regulator responsible for regulating the transcription and expression of multiple antioxidant stress protein genes, including superoxide dismutase (SOD), heme oxygenase-1 (HO-1), and γ-glutamylcysteine synthetase (γ-GCS) [13]. Keap1 is the main intracellular regulator of Nrf2 with five domains, each inhibiting Nrf2 activity. The antioxidant response element (ARE) is a specific promoter sequence located upstream of protective genes (SOD, GST, etc.), and Nrf2 is its activator. Under basal conditions, Keap1 binds Nrf2 to maintain low transcriptional activity. Upon attack by ROS or highly oxidizing substances, Keap1 dissociates from Nrf2, and activated Nrf2 enhances the transcription of antioxidant proteins by binding to ARE. The Nrf2-NF-κB axis crosstalks to coordinately regulate cellular redox homeostasis and inflammation. Numerous studies show that Nrf2 antagonizes NF-κB-driven inflammation, so modulating Nrf2 alleviates oxidative and inflammatory damage[13].

Activation of Nrf2 depends on its dissociation from Keap1, mainly through two mechanisms[14]((*e.g.*, Figure 5): ① Electrophiles and ROS modify Keap1 cysteine residues, inducing conformational changes and Nrf2 dissociation; ② Multiple kinases (PKA, PKC, MAPKs, PI3K) phosphorylate Nrf2 to regulate its transcriptional activity. In high glucose conditions, suppressed Gs pathway reduces PKA activity, impairing Nrf2 phosphorylation and inhibiting Nrf2-Keap1 uncoupling, thereby downregulating Nrf2-dependent antioxidant proteins (HO-1, GCLC, etc.) and weakening free radical scavenging. Meanwhile, excessive glucose is metabolized through the tricarboxylic acid cycle, and electrons generated are passed along the electron transport chain to produce ROS, leading to excessive ROS production in the body.. Nrf2 deficiency activates NOX2, further promoting ROS accumulation. Nrf2 knockout mice exhibit reduced antioxidant protein expression and enhanced oxidative damage.

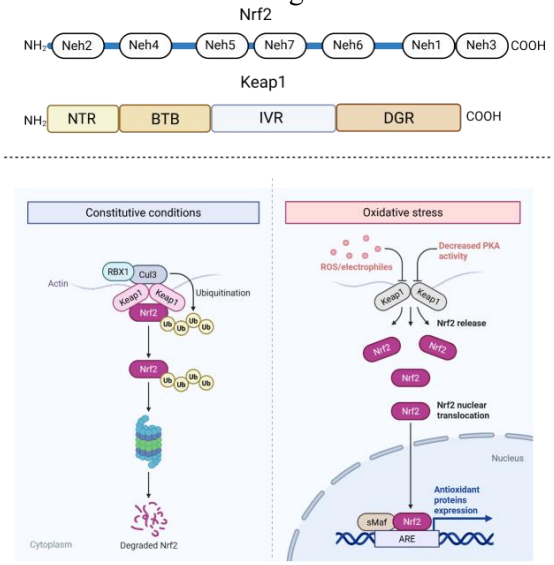

Figure 5. Regulatory Mechanism of the Gsα Pathway on Nrf2 under High-Glucose Conditions (Left: Normal Condition / Right: High-Glucose Condition)

Excessive ROS attacks retinal capillaries, tissue, and mitochondria. ROS-induced capillary damage increases permeability and leukocyte infiltration, aggravating inflammation. Retinal tissue has a high content of unsaturated fatty acids and efficient glucose uptake and utilization, which generate large amounts of reactive oxygen species that attack retinal tissue and induce oxidative stress [15]. Mitochondrial

DNA is susceptible to oxidative damage, causing mutations and amplified oxidative stress; inner membrane damage impairs the electron transport chain and metabolic enzymes; ROS reduce mitochondrial membrane potential, triggering cytochrome C release and apoptotic signaling [15].

In high glucose, the Gs-cAMP-PKA-mediated Nrf2 pathway is inhibited, reducing antioxidant capacity and causing excessive ROS that induces retinopathy.

GSK3β is a pro-apoptotic factor inducing apoptosis in cardiomyocytes and tumor cells. GSK3β promotes apoptosis by [16] (e.g., Figure 6): ① Phosphorylating p53 at Ser33 in mitochondria, facilitating p53 translocation to mitochondria, binding and inactivating Bcl2, and promoting Bax oligomerization; ② Inducing Bax conformational change and mitochondrial translocation, increasing outer membrane permeability and cytochrome C release. Studies have shown that phosphorylation at Tyr279/Tyr216 can activate the activity of GSK3β, whereas phosphorylation at Ser21/Ser9 can inhibit the activity of GSK3β [17] .PKA inhibits apoptosis by phosphorylating Ser9 of GSK3β. In high glucose, Gs pathway suppression reduces PKA activity, relieving GSK3β inhibition and activating apoptotic signaling.

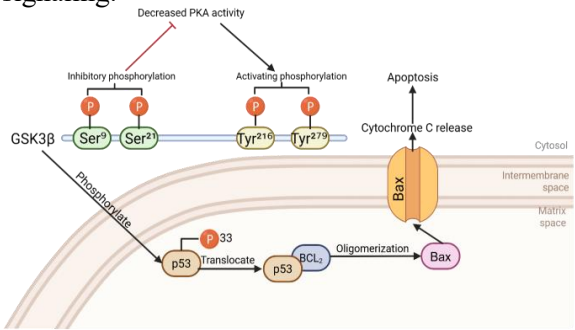

Figure 6. Regulatory Mechanism of the Gsα Pathway on GSK3β under High-Glucose Conditions

4. Research Progress of the Gs Protein Pathway as a Therapeutic Target for DR

4.1 Intervention Effects of Traditional Pathway Agonists

Classic agonists targeting the Gs pathway for DR include GLP-1, adenosine, and β-adrenergic receptor (β-AR) agonists, which protect the BRB, suppress inflammation and oxidative stress, reduce apoptosis, and ultimately preserve the neurovascular unit.

4.1.1 Glucagon-like Peptide-1 Receptor (GLP-1R) Agonists

Glucagon-like peptide-1 receptor (liraglutide, semaglutide, dulaglutide, exenatide) are the most clinically advanced agents. GLP-1 promotes β-cell proliferation and survival, enhances insulin secretion, and inhibits glucagon secretion[18]. Binding to its receptor activates the cAMP/PKA/PI3K pathway: cAMP induces extracellular Ca^{2+} influx, while PKA promotes the closure of ATP-sensitive potassium channels and simultaneously activates the opening of L-type Ca^{2+} channels, elevating intracellular Ca^{2+} to enhance insulin exocytosis, lowering blood glucose and reducing high glucose-induced retinal damage[18]. GLP-1 may inhibit β-cell apoptosis via PI3K/Akt and MAPK pathways, regulating pro- and anti-apoptotic proteins. It also suppresses gastric acid secretion, appetite, and gastric emptying to control postprandial glucose.

4.1.2 Anti-VEGF Agents

As first-line clinical drugs, anti-VEGF agents (ranibizumab, conbercept, aflibercept) can delay visual impairment and improve long-term therapeutic effects via intravitreal injection.. However, they require intravitreal injection, increasing infection risk, and repeated injections may cause complications (cataract, elevated intraocular pressure, endophthalmitis) [19].

4.2 Novel Candidate Drugs/Molecules/ Techniques

Emerging techniques for DR include laser photocoagulation and vitrectomy, minimizing damage to healthy tissue and preserving retinal structure. Anti-VEGF agents are widely used due to rapid onset and favorable prognosis. Corticosteroids serve as adjuvant therapy, alleviating laser-induced adverse effects but carrying risks of tolerance[19].

5. Current Research Gaps and Future Directions

5.1 Major Limitations of Existing Studies

Although GPCRs are critical in DR, studies on the specific mechanism of G protein regulation are fragmented, lacking a systematic regulatory network. Key targets and mechanisms of the Gs pathway in DR remain undefined. DR research is largely limited to in vitro and animal models, with insufficient clinical samples (retinal tissue,

aqueous humor, vitreous) hindering clinical translation. Current treatments are effective only for advanced DR, lacking targeted therapies for early and middle stages. Crosstalk between the Gs pathway and other signaling axes requires further clarification.

5.2 Key Future Research Directions

Future studies should use single-cell sequencing and proteomics to identify core targets and establish a comprehensive regulatory network. Clinical sample collection should be expanded to detect Gs pathway-related molecules (cAMP, Gαs) in aqueous/vitreous humor at different DR stages, identifying early diagnostic biomarkers. Specific agonists should be developed targeting stage-dependent Gs pathway mechanisms. Crosstalk between the Gs pathway and other signaling axes should be elucidated to enable combination therapy. Neurovascular unit protection should be explored to reduce long-term sequelae.

6. Conclusion

The Gs-cAMP-PKA pathway plays a vital role in DR progression, particularly in regulating inflammation, oxidative stress, apoptosis, and neurovascular unit protection. High glucose-induced inhibition of the Gs pathway exacerbates DR. Research on the Gs pathway provides novel therapeutic targets and strategies for early DR intervention. Traditional agonists have shown clinical efficacy, and Gs pathway-targeted agonists exhibit promising effects in preclinical models. Further investigation into core regulatory nodes and clinical validation will promote bench-to-bedside translation of Gs pathway-targeted therapies, offering new drugs and strategies for early diagnosis and treatment of DR.

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