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Retinal ischemic diseases and promising therapeutic molecular targets

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Summary. Retinal ischemia is a fundamental pathologic condition associated with retinal vascular occlusion, glaucoma, diabetic retinopathy, age-related macular degeneration, and other eye diseases. Extensive inflammation, redox imbalance, apoptosis, and abnormal vascular formation in retinal ischemia could lead to visual impairments. Developing or finding effective treatments is urgently needed to protect the eye against retinal ischemia and related damage. To address the demand, we have searched for promising therapeutic molecular targets in the eye (e.g., hypoxia-inducible factor [HIF], peroxisome proliferator-activated receptor-alpha [PPARα], and nicotinamide adenine dinucleotide [NAD^+]), and found that modulations of each molecular target might protect the eye against retinal ischemic damage in terms of complex pathologic mechanisms. In the current article, we review and update the therapeutic evidence of modulation of HIF, PPARα, or NAD^+ and discuss future directions for developing promising drugs based on these molecular targets. This summary urges research to obtain more solid evidence of each molecular target in retinal ischemic diseases.

Key words: Hypoxia-inducible factor; Nicotinamide adenine dinucleotide; Peroxisome proliferator-activated receptor-alpha; Retinal ischemia

Introduction

Retinal ischemia occurs when the circulation of the retina is insufficient to meet its metabolic demands. As the retina is one of the most oxygen-demanding tissues in our body (Ames, 1992, Joyal et al., 2018), failure of the oxygen supply can directly affect retinal metabolic status.

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The retina has a dual blood supply. The outer retina, which mainly contains photoreceptors, is nourished indirectly by the choriocapillaris (Behar-Cohen et al., 2020; Lejoyeux et al., 2022). The inner retina, where the other retinal cell types reside, is fed by branches of the central retinal artery derived from the ophthalmic artery (Michalinos et al., 2015). From a structural point of view, the outer retina has the possibility of being non-ischemic following occlusion(s) of the retinal artery. However, ischemia could occur in the outer retina in the case of abnormal choriocapillaris or by dysfunction of the retinal pigment epithelium (the other case of pathologic conditions in the eye) (McLeod et al., 2009; Ţălu and Nicoara, 2021).

Extreme intraocular pressure can also induce retinal ischemia. Retinal ischemia/reperfusion (I/R) plays a role in various retinal ischemic conditions, such as diabetic retinopathy (DR), glaucoma, and vascular ischemic retinopathy (Hartsock et al., 2016; Lee et al., 2022b). This type of injury may trigger pathological processes, including generating reactive oxygen species and retinal inflammation, which can ultimately lead to the death of retinal neuronal cells (Ghiardi et al., 1999; Minhas et al., 2016). In a previous study with mice, we elevated the intraocular pressure to 100 mmHg and observed the whitening of retinal vessels, confirming the induction of ischemia (Cakir et al., 2023). Given the lack of effective treatments currently available for the death of retinal ganglion cells, ongoing preclinical research aims to identify promising neuroprotective medications that could potentially prevent and mitigate the effects of retinal I/R injury.

DR is recognized as a pathology where ischemia is a crucial factor. Ischemia in the retina initiates neovascularization, which can subsequently lead to secondary complications such as hemorrhage and retinal detachment (Kollias and Ulbig, 2010; Cruz-Iñigo et al., 2014; Shaikh et al., 2023). However, inducing diabetes in mice does not necessarily result in neovascularization, failing to replicate the human condition of DR accurately. Consequently, an oxygen-induced retino-

pathy (OIR) model has become widely utilized to induce ischemia and subsequent neovascularization in the retina (Smith et al., 1994; Tomita et al., 2019, 2021). Similarly, a unilateral common carotid artery occlusion model is also considered valuable for inducing retinal ischemia, offering a relevant perspective (Lee et al., 2020a). These experimental models are crucial for understanding the mechanisms of retinal ischemia and developing potential treatments for conditions partly like DR.

Taken together, retinal ischemia can occur in various types of retinal diseases. Therefore, developing or finding effective treatments is essential to protect the eye against retinal ischemia and related damage. In this review article, we introduce three promising therapeutic molecular targets (hypoxia-inducible factor [HIF], peroxisome proliferator-activated receptor-alpha [PPARα], and nicotinamide adenine dinucleotide [NAD^+]) for retinal ischemic diseases based on our research experience (Fig. 1).

Main body

Hypoxia-inducible factor (HIF) inhibition

HIF is a potent intracellular regulator responsive to hypoxia, playing a significant role in erythropoiesis, angiogenesis, cell proliferation, death/survival, and metabolism. HIF is a heterodimeric complex transcription factor composed of an oxygen-dependent subunit, HIF-1α, and a constitutively active nuclear subunit, HIF-1β (Ke and Costa, 2006). Under normoxic conditions, HIF-1α is rapidly degraded by the ubiquitin-proteasome pathway (Hong et al., 2004). The von Hippel-Lindau (VHL) protein binds to the hydroxylated HIF-1α via prolyl hydroxylase (PHD) and induces polyubiquitination and degradation. Under hypoxic conditions, PHD function is decreased, leading to the stabilization of HIF-1α and subsequent translocation into the nucleus. Upon translocation, HIF-1α binds to HIF-1β, inducing expressions of hypoxia-responsive genes, such as vascular endothelial cell growth factor (VEGF), Bcl-2 interacting protein 3 (BNIP3), pyruvate dehydrogenase kinase 1 (PDK1), angiopoietin-like 4 (ANGPTL4), endothelin-1 (ET1), or heme oxygenase-1 (HO-1) (Lee et al., 2022a). Lack of oxygen and subsequent hypoxic cellular responses implicate HIF in several ocular pathologies.

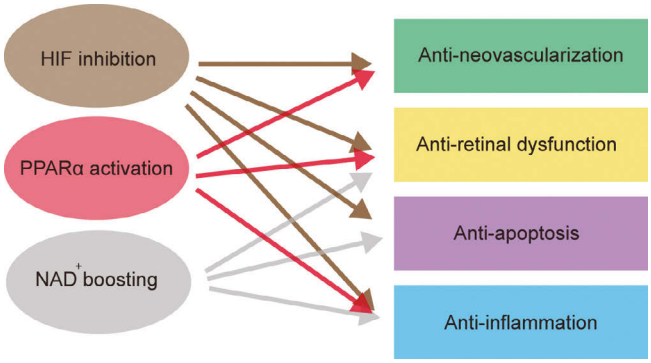

Fig. 1. A summary of the current evidence on modulating hypoxia-inducible factor (HIF), peroxisome proliferator-activated receptor-alpha (PPARα), and nicotinamide adenine dinucleotide (NAD^+) for retinal ischemic diseases. Each arrow and color (HIF: brown; PPARα: light red; NAD^+: gray) indicates the relationship with each pathologic effect, such as anti-neovascularization, retinal dysfunction, apoptosis, and inflammation.

Over the last decade, many preclinical studies have focused on understanding the therapeutic roles of HIF-1α inhibition in ocular neovascularization. These studies have generally suggested pharmacologic HIF inhibitors (digoxin and acriflavine) to suppress ocular neovascularization. Digoxin is initially used to treat heart failure and abnormal heart rhythms and is responsible for reversible inhibition of the cell membrane sodium-potassium (Na^+/K^+) ATPase (Patocka et al., 2020; Obradovic et al., 2023). Yoshida et al. mainly found that digoxin suppressed retinal and choroidal neovascularization by reducing HIF-1α levels and blocking various pro-angiogenic signaling pathways (Yoshida et al., 2010). Acriflavine is known as a topical antiseptic fluorescent drug (Piorecka et al., 2022). Zeng et al. demonstrated that intravitreous or intraperitoneal injection of acriflavine suppressed retinal and choroidal neovascularization (Zeng et al., 2017). Intraocular injection of acriflavine reduced retinal HIF-1-responsive gene expressions. Suprachoroidal injection of acriflavine further suppressed choroidal neovascularization. Digoxin and acriflavine seem to be good therapeutics for anti-neovascularization in the eye. However, side effects related to toxicities have emerged: 1) acriflavine accumulation in the retina inhibits retinal function (Zhang et al., 2023) and 2) digoxin histologically causes severe retinal degeneration (Hinshaw et al., 2016). In this regard, Zhang et al. aimed to develop an HIF inhibitor structurally unrelated to acriflavine (termed "32-134D") and found that 32-134D was not toxic to the retina yet could inhibit HIF accumulation and normalize HIF-responsive gene expression in mice and human retinal organoids (Zhang et al., 2023).

We focused on natural products and their essential chemical components to find novel HIF inhibitors without side effects (Lee et al., 2022a). A fairy chemical 2-azahypoxanthine (AHX) derived from mushrooms and fish ingredients from *Decapterus tabl* suppressed retinal neovascularization as novel HIF inhibitors (Lee et al., 2020b; Shoda et al., 2020). Garcinia cambogia extract with hydroxycitric acid, lactoferrin, and rice bran with vitamin B6 were found to be novel HIF inhibitors and suppress choroidal neovascularization in mice (Ibuki et al., 2019, 2020a,b). A superfood Camu-Camu (*Myrciaria dubia*) could have HIF-inhibiting effects in ARPE-19 cells (Nakai et al., 2023). Dramatic systemic damage was not found during the experimental stage. Taken together, targeting HIF-1α could be a good therapeutic strategy to treat retinal ischemic diseases.

Clinical point of view on HIF inhibition

VEGF has been identified as a critical factor for DR progression. It has been reported that HIF-1α protein levels in the human vitreous increased in proliferative diabetic retinopathy (PDR) patients, and its levels could decrease after laser treatment when ischemia is resolved (Wert et al., 2016). This mechanism underscored the presumed involvement of HIF-1α in the pathogenesis of DR. A research group showed HIF-1α and HIF-2α expression in the mortal human retina, and the findings of this study indicated an upregulation of HIF-1α and, to a lesser extent, HIF-2α expression within the inner nuclear layer (INL) of the ischemic retinas under conditions of retinal neovascularization. They suggested that both HIF-1 and HIF-2 may contribute to the upregulation of angiogenic genes (e.g., *VEGF* and *ANGPTL4*), potentially facilitating the development of retinal neovascularization in patients with sickle cell disease (Zhang et al., 2021).

Another group has shown that the HIF-1α Pro582Ser polymorphism might play a protective role against the development of severe DR. This polymorphism confers relative resistance to hyperglycemia-mediated repression of HIF-1α, potentially safeguarding against DR progression. The study suggested that individuals carrying the Pro582Ser mutation may have a reduced risk of developing severe DR, even after adjusting for known risk factors like the duration of diabetes, levels of hyperglycemia, and hypertension (Ekberg et al., 2019). These findings elucidated the significant role of HIF, particularly HIF-1α, in the pathogenesis and progression of DR. However, another group indicated that genetic variations in HIF-1α did not exhibit a direct correlation with the onset of diabetes or its complications, such as DR (Liu et al., 2021). The genetic polymorphism associated with HIF-1α not only provides insight into the mechanisms underlying DR but also may suggest potential therapeutic targets for preventing or mitigating the progression of this condition. The modulation of HIF-1α activity and its downstream factors could be crucial in developing new strategies for the treatment of DR.

Peroxisome proliferator-activated receptor-alpha (PPARα) activation

PPARα is one of the important nuclear receptor PPAR families (PPARα, PPARδ, and PPARγ). PPARα has been found to regulate various gene expressions in lipid and carbohydrate metabolism (Tajnšek et al., 2020; Lin et al., 2022). PPARα has been suggested to be expressed in many types of cells in the skeletal muscle, heart, liver, brown adipose, kidney, intestinal mucosa, adrenal gland, retina, retinal pigment epithelium, and vasculature, such as endothelial cells, smooth muscle cells, monocytes, and macrophages (Tyagi et al., 2011; Tomita et al., 2020a; Escandon et al., 2021). The activation of PPARα using fenofibrate and pemafibrate was found to increase circulating levels of high-density lipoprotein cholesterol or decrease serum levels of triglycerides, which could improve lipid conditions in our body to exert therapeutic roles on inflammation and insulin resistance under pathologic metabolic disease conditions (Zandbergen and Plutzky, 2007; Katsiki et al., 2013). Furthermore, accumulated evidence suggested that PPARα activation can be a strong therapeutic molecular target in ischemic retinopathies (Lee et al., 2023b).

In our murine model of common carotid artery occlusion-induced retinal ischemia, oral administration of fenofibrate or pemafibrate protected against retinal dysfunction (Lee et al., 2021a,b). In a murine model of retinal I/R injury through transient elevation of intraocular pressure, oral administration of pemafibrate could protect against retinal inner retinal damage (Lee et al., 2022c). Yao et al. also showed that PPARα activation by fenofibric acid treatment could promote the survival of retinal ganglion cells, mitigate thinning of the ganglion cell complex, and decrease the latency of positive waves of flash visual-evoked potentials after I/R injury (Yao et al., 2021).

Regarding diabetic conditions, we found that pemafibrate administration could show protective effects against retinal dysfunction in a murine model of STZ-induced DR (Tomita et al., 2020b). Suto et al. suggested that pemafibrate treatment could reduce plasma eicosanoid levels and ameliorate endothelial dysfunction under the diabetic condition (Suto et al., 2021). Shiono et al. found that pemafibrate treatment could inhibit diabetes-induced vascular leukostasis and leakage through the upregulation of *THBD* encoding thrombomodulin (Shiono et al., 2020). Tanaka et al. also found protective effects of pemafibrate treatment against DR-induced injury in spontaneously diabetic Torii fatty rats (Tanaka et al., 2024).

Regarding ocular neovascularization, we found that pemafibrate treatment could suppress choroidal and retinal neovascularization in adult and young mice (Tomita et al., 2019; Lee et al., 2023c). Gong et al. suggested that fenofibrate could inhibit cytochrome P450 epoxygenase 2C activity to suppress pathological ocular angiogenesis (Gong et al., 2016). Zhao et al. found that fenofibrate could inhibit the expression of VEGFC and VEGFR-3 in retinal pigment epithelial cells exposed to hypoxia (Zhao et al., 2015). Qiu et al. used fenofibrate-loaded biodegradable nanoparticles to improve disease conditions of DR and neovascular age-related macular degeneration (Qiu et al., 2019). The increasing preclinical evidence suggests that PPARα activation might be an excellent molecular therapeutic target for retinal ischemic diseases.

Clinical point of view on PPARα activation

Clinical trial for fenofibrate

In prior research, the Fenofibrate Intervention and

Event Lowering in Diabetes (FIELD) and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) studies have demonstrated that fenofibrate (a PPARα activator) could diminish the necessity for laser treatments and slow down the advancement of DR (Keech et al., 2005; Group et al., 2010). Fenofibrate has been extensively investigated across various diseases, with current studies and clinical trials further exploring its benefits. Specifically, fenofibrate has shown promise in treating eye conditions such as DR and diabetic macular edema (DME) (Lee et al., 2023a).

In a recent database study, Meer et al. surveyed 150,252 patients with non-proliferative diabetic retinopathy (NPDR), assessing the effects of fenofibrate, a lipid-lowering agent prescribed to 3.9% (5,835 patients) of the cohort, between 2002 and 2019 (Meer et al., 2022). They found that fenofibrate use was associated with a decreased risk of developing vision-threatening diabetic retinopathy (VTDR) and PDR, with hazard ratios (HR) of 0.92 and 0.76, respectively, indicating its potential protective effects. However, no significant impact was observed on DME. These findings suggested the potential of fenofibrate in reducing the risk of severe DR forms, highlighting the need for further clinical trials to explore a causal relationship.

Clinical trials are currently exploring fenofibrate's potential in treating ischemic eye diseases, with three notable studies underway. The Protocol AF Trial is a randomized clinical trial with 560 participants with type 1 or type 2 diabetes, focusing on preventing the worsening of DR over six years. It targets patients with mild to moderately severe NPDR without center-involved diabetic macular edema (CI-DME), aiming to establish fenofibrate as a preventive treatment against progression to PDR. This trial emphasizes a collaborative care approach between ophthalmologists and primary care providers for fenofibrate's safe prescription and monitoring, exploring the impact of blood glucose level fluctuations on DR outcomes (ClinicalTrials.gov identifier: NCT04661358).

The FAME 1 EYE Trial is a study on adults with type 1 diabetes mellitus with NPDR, examining the effectiveness of 145 mg of daily fenofibrate in preventing eye damage over an average of 36 months. This trial aims to validate fenofibrate's potential to reduce the risk of blindness in type 1 diabetes, building on evidence of its benefits in 450 type 2 diabetes individuals (ClinicalTrials.gov identifier: NCT01320345). The study will evaluate the occurrence of a two-step progression in the ETDRS score, the presence of clinically significant macular edema, and the necessity for interventions such as laser surgery, intraocular anti-VEGF or corticosteroid therapy, or vitrectomy, specifically adjudicated for DR.

The LENS Trial is a phase 4, multicenter, randomized, placebo-controlled study assessing fenofibrate's roles in slowing or preventing DR and maculopathy in approximately 1,060 diabetes patients over four years. The trial utilizes NHS Scotland registries for outcome and safety data, focusing on fenofibrate's efficacy in managing referable DR and maculopathy (ClinicalTrials.gov identifier: NCT03439345). These trials collectively aim to provide conclusive evidence on fenofibrate's roles in preventing and managing diabetic eye complications, potentially offering a less invasive and cost-effective treatment alternative.

Clinical trial for pemafibrate

Pemafibrate exhibits a higher affinity for PPARα than fenofibrate (Tomita et al., 2020a). The PROMINENT Phase 3 trial was initiated to assess pemafibrate's capacity to mitigate cardiovascular (CV) event risks among type 2 diabetes mellitus patients undergoing statin therapy. The trial aimed to measure the incidence of a set of severe CV occurrences, including non-fatal myocardial infarction, non-fatal ischemic stroke, coronary reperfusion, and cardiovascular mortality. Despite the absence of significant safety issues, the trial was discontinued due to its interim results, which indicated that the primary outcome was unlikely to be achieved. Nonetheless, this investigation yielded valuable insights into potential benefits in emerging areas of treatment for non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), warranting further exploration (ClinicalTrials.gov identifier: NCT03071692).

Unfortunately, the PROMINENT-Eye Ancillary Study, aimed at exploring the effects of pemafibrate on DR progression, was halted early because it did not recruit a sufficient number of participants to meet its research criteria (ClinicalTrials.gov identifier: NCT03345901). Clinical data are required to know the effect of this disease.

Recently, an extended-release version of pemafibrate was launched in Japan. A clinical trial has been actively investigating the long-term efficacy and safety of once-daily morning or evening dosing of pemafibrate extended-release for treating dyslipidemia over 52 weeks. This multicenter, randomized, open-label, and parallel-group phase III study focuses on patients with dyslipidemia, particularly those with high triglyceride levels. A total of 110 patients with dyslipidemia were administered extended-release pemafibrate at a dosage of 0.2 mg/day, either in the morning or evening. Having completed this, the study anticipates favorable outcomes (ClinicalTrials.gov identifier: NCT04716595).

Nicotinamide adenine dinucleotide (NAD^+) boosting

NAD^+ is an essential molecule in many metabolic pathways, acting as a co-factor for NAD^+-dependent enzymes (Covarrubias et al., 2021). NAD is crucial in glycolysis, the tricarboxylic acid cycle (TCA cycle), and oxidative phosphorylation. It acts as an electron carrier, oscillating between its oxidized form (NAD^+) and its

reduced form (NADH). This electron transfer is fundamental to generating ATP, which provides energy for various cellular activities. Nicotinamide phosphoribosyl transferase (NAMPT) is the rate-limiting enzyme in the mammalian NAD^+ biosynthesis salvage pathway, converting nicotinamide to nicotinamide mononucleotide (NMN). The NMN generated is then converted to NAD^+ by nicotinamide mononucleotide adenylyltransferase, NMNAT (Fig. 2). Accumulated evidence showed that NAD^+ levels decreased with aging (McReynolds et al., 2020). Therefore, NAD^+ boosting has been suggested as an attractive method to protect against age-related diseases.

Based on our research outcomes, NMN, a direct precursor of the vital molecule (NAD^+) (Lee et al., 2024), could protect against retinal damage in murine models of unilateral carotid artery occlusion or retinal I/R injury (Lee et al., 2022b,d). Other groups' studies showed that nicotinamide treatment might provide neuroprotection in glaucoma by protecting against mitochondrial and metabolic dysfunction (Williams et al., 2017; Tribble et al., 2021). However, only a few recent papers related to NAD+ boosting have reported on ischemic retinopathies. Much larger experimental studies to examine how NAD^+ boosting could prevent or protect against retinal ischemia-induced impairments in the eye should be conducted to make a reasonable conclusion.

Clinical point of view on NAD^+ boosting

Evidence showed that reductions in NAD^+ levels were detected in aging (McReynolds et al., 2020), and changes in serum NAMPT levels were associated with several diseases, such as acute lung injury, chronic kidney disease, and rheumatoid arthritis (Ye et al., 2005; Axelsson et al., 2007; Senolt et al., 2011). Furthermore, NAD^+ metabolism plays a pivotal role in the pathophysiology of ocular disorders, participating in the progression of various ocular diseases (Lee et al., 2024). It has been reported that patients with PDR have much lower levels of NAD^+/NADH in the vitreous compared with normal individuals (Choudhuri et al., 2013).

As in DR, retinal vein occlusion (RVO) also has an aspect of ischemia. Kaja et al. revealed a marked reduction in serum NAMPT levels in individuals with a history of RVO, compared with healthy controls (Kaja et al., 2015). They suggested that NAMPT levels in RVO may become one of the biomarkers of this disease; nonetheless, more clinical evidence is needed.

Although it is not directly related to retinal ischemia, the Nicotinamide in Glaucoma trial (NCT05405868) focuses on examining the therapeutic effects of nicotinamide in patients with open-angle glaucoma. Regarding NAD^+ boosting, promising outcomes are expected (Bhartiya, 2022). Another study reported that *NMNAT1* mutations induced severe neonatal neurodegeneration of the central retina and early-onset optic atrophy in 22 individuals. Their clinical manifestations aligned with Leber congenital amaurosis (LCA), suggesting that these mutations may impair the neuroprotection of photoreceptor cells (Perrault et al., 2012). Another group showed that sequencing of *NMNAT1* in 284 families with LCA revealed 14 rare mutations in 13 additional affected individuals. These findings represented the first association of an NMNAT isoform with a disease condition and indicated that mutations in *NMNAT1* are causative of LCA (Falk et al., 2012). However, the causal relationship between the decrease in NAMPT levels and eye diseases remains undetermined, necessitating further comprehensive investigation to elucidate the underlying mechanisms.

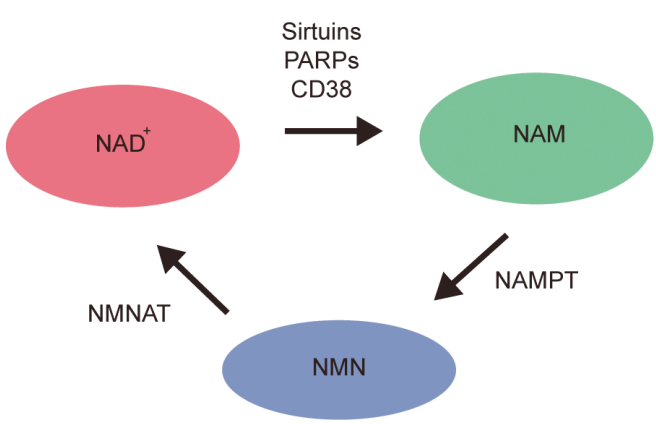

Fig. 2. The NAD^+ biosynthetic pathway. Nicotinamide phosphoribosyltransferase (NAMPT) is an enzyme within the NAD^+ biosynthetic pathway, responsible for converting nicotinamide (NAM) to nicotinamide mononucleotide (NMN). NMN is converted to NAD^+ by nicotinamide mononucleotide adenylyltransferase (NMNAT). CD38, poly ADP-ribose polymerases (PARPs), or sirtuins might be involved in the conversion of NAD^+ into NAM.

Other important molecular targets

Other than targeting HIF, PPARα, or NAD^+, many researchers have aimed to find promising molecular targets to treat retinal ischemic diseases: microRNAs, pathological inflammatory markers, and pro- or anti-apoptotic markers (Dunaief et al., 2002; ElShelmani et al., 2021; Guo et al., 2022).

In particular, a recent report conducted by Crespo-Garcia et al. suggested that pathological ocular angiogenesis might be associated with cellular senescence, and the inhibition of B-cell lymphoma-extra large (BCL-xL) could eliminate senescent cells under the disease condition, finally reducing ocular neovascularization (Crespo-Garcia et al., 2021). Bcl-xL, of the Bcl-2 family (Adams and Cory, 1998), is a well-known molecule that exerts anti-apoptotic properties in various tissues (González-García et al., 1994). Its mode of action has been suggested to regulate cell death by controlling mitochondrial membrane permeability and the release of cytochrome c (Shimizu et al., 1999). Subsets of endothelial cells having senescence features were no longer seen in the BCL-xL-inhibitor-treated

retina, which could make the retina favorable to undergo physiological repair of the vasculature (Crespo-Garcia et al., 2021). Ryu et al. also demonstrated that the Bcl-2/Bcl-xL inhibitor could attenuate retinal degeneration via the selective regulation of senescent RPE cell death (Ryu et al., 2023). Taken together, its modulation could be another promising strategy to treat retinal ischemic diseases.

Future directions and conclusions

In this review article, we have updated the therapeutic evidence of the modulation of HIF, PPARα, or NAD^+ in retinal ischemic diseases. Each molecular target seems to have important implications in terms of therapeutic effects in retinal ischemic diseases. Then, what is the relationship between these three molecular

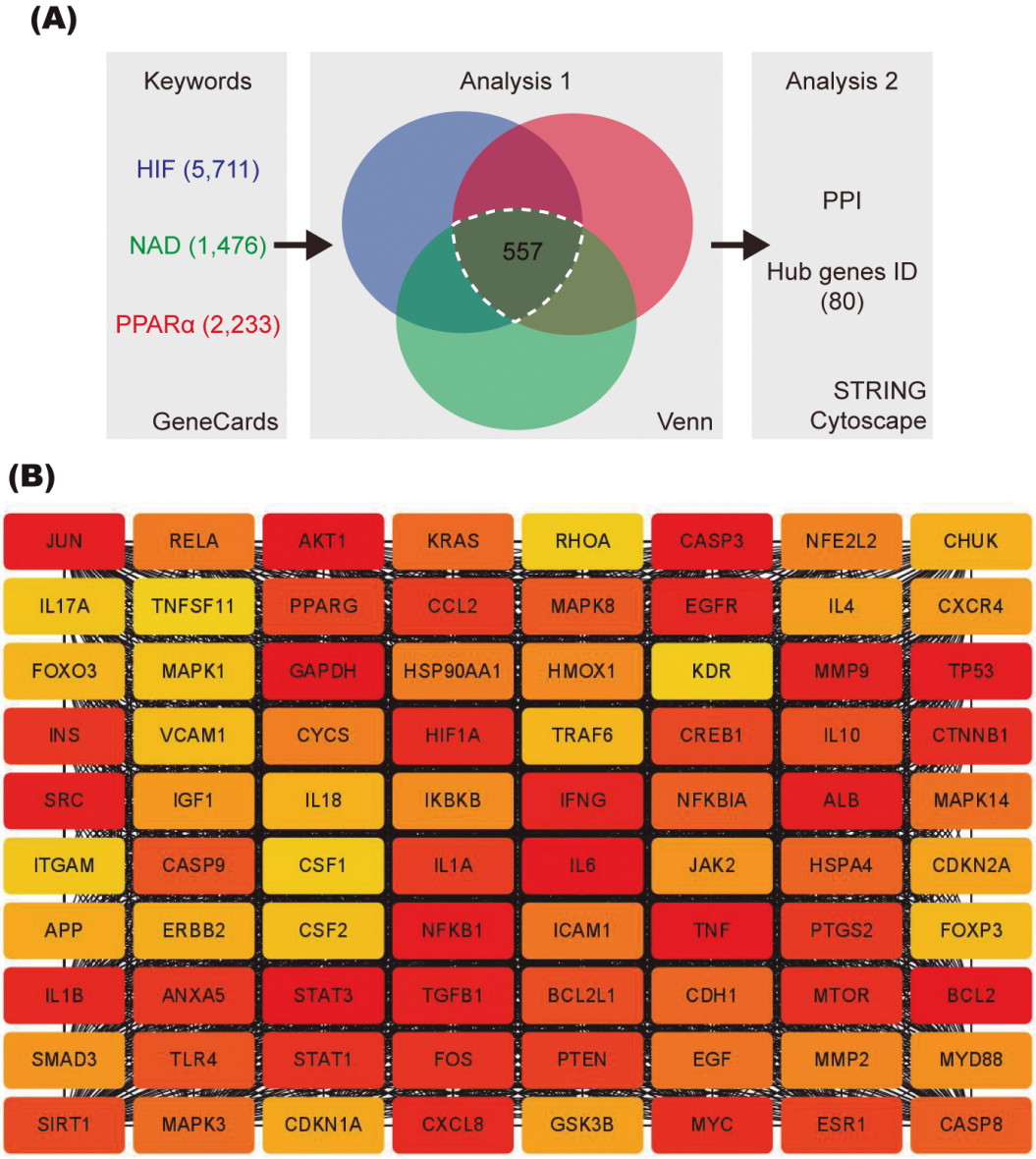

Fig. 3. Screening for potential genes affected by the modulation of hypoxia-inducible factor (HIF), peroxisome proliferator-activated receptor-alpha (PPARα), and nicotinamide adenine dinucleotide (NAD^+) through bioinformatic analyses. **A.** A flow chart of bioinformatic analyses. GeneCards (www.genecards.org) was used to collect genes associated with HIF, PPARα, or NAD^+: 5,711 genes for HIF, 1,476 genes for NAD, and 2,233 genes for PPARα (searched in March, 2024). Venn diagram shows that 557 genes overlapped. After 557 genes were uploaded to STRING (https://string-db.org/) to examine protein-protein interaction (PPI) networks, the open-source software platform Cytoscape (https://cytoscape.org/) was used to visualize complex PPI networks of the genes from STRING. Hub genes predicted to have important nodes and subnetworks were identified by the CytoHubba, a useful plug-in of Cytoscape. Node scores (the highest degree of connectivity) were ranked by the maximum clique centrality (MCC) method in the CytoHubba. Venn: Venn diagram; PPI: protein-protein interaction; ID: identification. **B.** The top 80 genes were selected for display (selected by the CytoHubba with the MCC method). A high degree of connectivity was presented (low to high: yellow to red).

targets? There has not been a direct research outcome yet regarding this aspect, however, there share points in common between various associated genes (Fig. 3), based on text data mining from GeneCards (www.genecards.org) with further interaction analyses via STRING (https://string-db.org/) and visualization using CytoHubba and Cytoscape (https://cytoscape.org/) (Shannon et al., 2003; Chin et al., 2014; Stelzer et al., 2016; Safran et al., 2021; Szklarczyk et al., 2021; Lee et al., 2022c).

Each keyword (HIF, NAD, or PPARα) was searched in GeneCards, and keyword-related genes were obtained (HIF: 5,711, NAD: 1,476, and PPARα: 2,233). Overlapped genes were collected from them and 80 Hub genes were further selected by the CytoHubba, as in our previous publication (Lee et al., 2022c). As a result, we found that many genes/pathways could be involved in targeting the HIF, NAD, or PPARα molecule.

The notion that outcomes are directly associated with retinal ischemic diseases needs further evaluation; for instance, CCL2 (monocyte chemoattractant protein 1) was suggested to increase under retinal ischemic conditions (Davies et al., 2006; Shahror et al., 2024). Its increase could attract many types of pro-inflammatory cells to the retina, finally leading to retinal degeneration. Toll-like receptor 4 (TLR4) could contribute to retinal I/R injury-induced damage (Dvoriantchikova et al., 2010). JUN is an important molecule for ocular hypertension-induced retinal ganglion cell damage (Syc-Mazurek et al., 2017). BCL2 family members, including BCL2, generally control the death of retinal ganglion cells (Maes et al., 2017). Therefore, understanding these genes and also others affected by modulations of these molecular targets could support further preclinical research and offer new possibilities for developing therapeutic interventions.

However, as clinical data are still lacking in this field, the accumulation of new clinical data will become increasingly important. Systemic molecular analyses from the preclinical studies are also recommended to obtain an overall picture of what is changing in damaged eyes. Our current summary urges further research to obtain more solid evidence of each or combined molecular targets in retinal ischemic diseases, which may help design future promising clinical studies.

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Conflict of interests. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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