• Review Article •

Regulatory factors of Nrf2 in age-related macular degeneration pathogenesis

Zi–Ling Hu[1], Yu–Xuan Wang[2], Zi–Yue Lin[3], Wen–Shuo Ren[2], Bo Liu[5], Hui Zhao[3,4], Qiong Qin[6]

[1]Five Year Program of Ophthalmology and Optometry 2019, Beijing Tong Ren Hospital, Capital Medical University, Beijing 100054, China
[2]Four Year Program of Traditional Chinese Pharmacy 2020, School of Traditional Chinese Medicine, Capital Medical University, Beijing 100069, China
[3]School of Traditional Chinese Medicine, Capital Medical University, Beijing 100069, China
[4]Beijing Key Laboratory of TCM Collateral Disease Theory Research, Beijing 100069, China
[5]Five Year Program of Ophthalmology and Optometry 2021, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China
[6]Biochemistry & Molecular Biology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China
Correspondence to: Qiong Qin. School of Basic Medical Sciences, Capital Medical University, No.10 Youanmen Wai Xitoutiao, Beijing 100069, China. qqin@ccmu.edu.cn
Received: 2023-07-24 Accepted: 2024-03-06

Abstract

● Age-related macular degeneration (AMD) is a complicated disease that causes irreversible visual impairment. Increasing evidences pointed retinal pigment epithelia (RPE) cells as the decisive cell involved in the progress of AMD, and the function of anti-oxidant capacity of PRE plays a fundamental physiological role. Nuclear factor erythroid 2 related factor 2 (Nrf2) is a significant transcription factor in the cellular anti-oxidant system as it regulates the expression of multiple anti-oxidative genes. Its functions of protecting RPE cells against oxidative stress (OS) and ensuing physiological changes, including inflammation, mitochondrial damage and autophagy dysregulation, have already been elucidated. Understanding the roles of upstream regulators of Nrf2 could provide further insight to the OS-mediated AMD pathogenesis. For the first time, this review summarized the reported upstream regulators of Nrf2 in AMD pathogenesis, including proteins and miRNAs, and their underlying molecular mechanisms, which may help to find potential targets *via* regulating the Nrf2 pathway in the future research and further discuss the existing Nrf2 regulators proved to be beneficial in preventing AMD.

● **KEYWORDS:** Nrf2; upstream regulators; retinal pigment epithelia; age-related macular degeneration; oxidative stress

DOI:10.18240/ijo.2024.07.21

Citation: Hu ZL, Wang YX, Lin ZY, Ren WS, Liu B, Zhao H, Qin Q. Regulatory factors of Nrf2 in age-related macular degeneration pathogenesis. *Int J Ophthalmol* 2024;17(7):1344-1362

INTRODUCTION

Age-related macular degeneration (AMD), which affects the macula and leads to deficit of central vision, is one of the main irreversible causes of severe visual impairment and blindness[1]. It is assessed that the number of AMD patients will reach 288 million by 2040 around the world[2], and 55.19 million by 2050 in China[3], which can be serious financial burden for the society. It is believed that AMD results from an interplay of genetic, environmental, and behavioral risk factors. Early-aged AMD, characterized by small drusens deposited and pigmentary alters at the rear pole of the fundus, will develop into wet AMD and dry AMD without timely and proper intervention[4]. Dry AMD accounts for 80%–90% of all AMD cases and can be treated by preserving retinal pigment epithelium (RPE) and photoreceptor cells. Neovascular AMD affects 10%–20% of AMD patients and can be treated by blocking neovascularization[5]. However, the treatment is still limited due to the deficient perception of molecular mechanisms underlying the pathogenesis of AMD, especially the initiation of AMD. The mechanisms of AMD pathogenesis are complicated and far from elusive. Currently, oxidative stress (OS) in RPE cells has been considered as a central contributor to AMD and its roles in the initiation and progression of AMD was firstly confirmed in humans in clnical trials[6-7]. The evaluation of this information highlights the central role that oxidative damage in the retina plays in contributing to major pathways, including inflammation and angiogenesis, found in the AMD phenotype[6].

RPE mediates the support function of photosensors and helps sustaining the homeostasis of subretinal space *via* multiple processes involving digestion of the photoreceptor outer

segments[8-9]. Reactive oxygen species (ROS)-induced damage of RPE cells is considered to be the initial pathological trigger of AMD occurrence[10] by inducing inflammation[11], mitochondria damage[12] and autophagy[13], which in turn further aggregate the injury of RPE cells and ultimately lead to the dysfunction or even atrophy of RPE cells[14]. The degeneration of photesensors occurs and leads to initiation of dry AMD. Then further recruitement of cytokines and chemokines promotes angiogenesis and choroidal neovascularization, results in wet AMD[15]. Increasing research has been performed to understanding the detailed mechniams involved in OS-mediated pathogenesis of AMD, especially the initiation of dry AMD due to its high prevalence. In recent years, nuclear factor erythroid 2 related factor 2 (Nrf2) is considered as a core player against OS by regulating the expression of multiple antioxidant proteins. Although Nrf2 does play a role in the pathogenesis of wet AMD, the study regarding to its involvement in choroidal neovascularization is limited and a few study was found[16]. Therefore, as a core player against OS, the role of Nrf2 in the development of OS-mediated pathogenesis of AMD has attracted more and increasing attention of researchers[17]. However, most studies paid attention to the role of Nrf2 and its downstream genes in AMD, despite the complex upstream regulation of Nrf2 activation. Multiple upstream proteins and kinases modulate the expression and activation of Nrf2, including key processes of ubiquitination, nuclear translocation, transcriptional activity and nuclear exportation of Nrf2[18]. This review summarized the current studies regarding to the factors that regulate Nrf2 expression thus influence Nrf2-mediated signaling pathways in the pathogenesis of AMD and the potential therapeutic drugs associated with these factors on AMD, and tried to present the current available studies regarding to the following questions: What regulators has been found to modulate Nrf2 expression in AMD or AMD models? How are non-coding RNAs involved in Nrf2 regulation? Do other drugs effect the activation of Nrf2 unintentionally? As more and more attention is focused on the therapeutic effects of various Nrf2 upstream inducers, this review might to some extent provide constructive suggestions for follow-up researches.

ROS-mediated Dysfunction and Degeneration of RPE Cells Plays a Central Role in the Progression of AMD

ROS is generated in many cellular process including aerobic respiration and nutrient metabolism, and it produces OS if not removed rapidly[19]. Compared with other tissues, retina is very prone to generate ROS and is more vulnerable to OS because of its high metabolism, highly perfusion[20] and frequent exposure to harmful environment[21-22]. Among the major constituent cells of the retina, RPE cells regulate the selective transportation between the choroid and the subretinal area, compensate the environmental changes in the subretinal space caused by hypermetabolism of photosensors and neurons[23-24] and help sustain visual cycles[25]. In short, the hyper-metabolism of RPE determines its susceptibility to OS, and thus RPE cells contain high concentrations of enzymatic and non-enzymatic anti-oxidants and keep the ability to repair oxidized lipids and damaged proteins and DNA to sustain the structural integrity and physiological functions of the retina[26]. However, the ability of RPE defensing against injury of oxidants diminishes with aging, making them display raised susceptibility to the cell damage induced by exogenic stressors[26].

OS in RPE cells induces oxidative damage to nuclear and mitochondrial DNA including strand breaks and DNA-protein cross links[27]. In contrast, the latter is more vulnerable to OS damage, as it located closer to sites where ROS is generated. Regions of the mitochondrial deoxyribonucleic acid (mtDNA) that are required for the transcription and replication of mitochondrial proteins are impacted by the mtDNA damage, which impairs respiratory complexes and mitochondria-nuclear signaling[28], engenders mitophagy, breaks the mitochondrial homeostasis and consequently increases the ROS production in RPE cells[27]. Besides, OS oxides enzymatic and structural proteins therefore influences multiple vital cellular metabolic process[29], and also mediates the peroxidation of lipids and lipoproteins, thus influences cellular autophagy[30]. The former give rise to the reduced autophagy activity by directly inactivating key lysosomal cysteine proteases and modificating substrate proteins that exerts competitive inhibition of lysosomal proteases[31-32]. While the latter induce a reduction of the fusion of lysosomes with phagosomes[30,33]. Lipoproteins can also trigger inflammation in RPE cells, including complement cascade dysregulation[30] and inflammasome activation[34], which have been proved to be the two vital immune factors in RPE[35-36]. The inflammation further raises the cells' susceptibility to OS damage, which forms a vicious loop and causes prolonged damage to the RPE cells[37]. All of these above ultimately lead to RPE cells progressive dysfunction and degeneration.

Therefore, dysregulation of RPE cells has been considered as an essential initiator in the mechanism of AMD development[38]. In histology, the normal cuboidal RPE becomes irregularly shaped and undergoes epithelial mesenchymal transition, and heterogeneous debris accumulates in Bruch's membrane, especially within the inner layer, leading to the degeneration of cones and rods and consequently results in visual loss[39-40]. The detachments of RPE layer and deposit of drusens compromise the integrity of elastic lamina and stimulate choroidal neovascularization (neovascular AMD)[41], while the flow of nutrients and clearance of waste products between

the choroicapillaris and RPE caused by drusens deposits is believed as an essential factor of atrophic AMD[38,42]. These waste products can prolong the exposure of RPE cells to the oxidizing extracellular environment and recruit many cytokines and chemokines and promote inflammatory response, angiogenesis and choroidal neovascularization, leading to wet AMD[43]. In conclusion, the destruction of RPE caused by OS plays an initial role in the onset and progression of AMD.

Nrf2 as a Pivot in AMD Nrf2 is a transcription factor encoded by NF-E2L2 containing 605 amino acids and being constituent of 7 conserved regions[44]. Increasing evidences have indicated its pivotal involvement in the progress of AMD[45-46]. Recently, Nrf2 is considered as a main redox sensor in regulating the cascade of antioxidant enzyme systems in RPE in the highly oxidative environment in dry AMD[47]. In RPE cells absent of Nrf2, AMD-like phenotype, including lipofuscin accumulation, drusens deposition and developing choroidal neovascularization, was observed[12,48] and can be alleviated by magnifying the endogenous expression of Nrf2 and its responsive antioxidant genes with electrophilic Nrf2 inducers or locally targeted antioxidant drugs[49]. Furthermore, additional studies in animal and cell models demonstrate that enhancing Nrf2 activation by drugs with antioxidative properties is a potential beneficial therapy for AMD as it promotes the protection for RPE cells from OS[50-51]. Together, these findings above illustrate a close connection between Nrf2 and the progress of AMD.

Direct anti-oxidant functions of Nrf2 ROS mediates the dysfunction and degeneration of RPE cells thus promotes the onset and progress of AMD. As a vital transcription factor in anti-oxidant responses, Nrf2 mainly senses the ROS levels and protects cells against OS via regulating the transcription of phase II detoxification enzymes and antioxidant proteins[52], including NAD(P)H:quinone oxidoreductase-1[53], heme oxygenase-1 (HO-1)[54], γ-glutamyl cysteine ligase catalytic subunit[55] and superoxide dismutase (SOD)[56]. Their anti-oxidant functions have been illuminated in detail in other tissues and cells before. Although their specific roles in RPE cells in physiological and pathological conditions have not been pointed in particular, here are some established evidences for their similar anti-oxidant functions in RPE cells. In aging RPE cells, high constitutive Nrf2 activity is observed, causing adaptive upregulation of anti-oxidant genes including NAD(P)H:quinone oxidoreductase-1 and HO-1 to provide adequate anti-oxidant protection for RPE cells under basal conditions[57]. Furthermore, a growing number of pharmacological studys indicate the benefit of activating Nrf2 and promoting the expression its downstream anti-oxidant genes in protecting RPE cells and treating AMD[58-59]. Treating H_2O_2-stimulated ARPE-19 cells (retinal pigment epithelial immortalized cell line from Amy Aotaki-keen eyes) with Aloperine, a quinolizidine alkaloid, increases the expression levels of nuclear Nrf2 and HO-1, enhances the activities of SOD and glutathione peroxidase in RPE cell line (ARPE-19 cells), thus greatly decreases the production of ROS and malondialdehyde and protected ARPE-19 cells from apoptosis[60]. Madecassoside treatment and quercetin-PC treatment also presents similar effects[56,61].

Indirect anti-oxidant functions of Nrf2 In addition to exerting direct anti-oxidant functions, Nrf2 also controls genes which are involved in mitochondria, autophagy and inflammation regulation indirectly *via* anti-oxidants from multiple pathways thus modulates the pathological processes in cells.

Nrf2 regulates the expression of mitochondrial transcription factor A, thus contributes to manage important physiological processes of mtDNA including its transcription, translation, and repair[62-63]. Furthermore, Nrf2 also accelerates the expression of alcohol dehydrogenase, aldehyde dehydrogenase and NADPH alenol oxidoreductase, consequently contributes to break down 4-hydroxynonenal modified proteins within electron transport chain complex I and II thus restores the normal function of mitochondrial electron transport chain[64]. Besides, Nrf2 upregulates the expression of phosphorylated 62-kDa protein (p62), a tyrosine phosphoprotein that facilitates the selective autophagic clearance of insoluble proteins in response to acute stress, at transcriptional level in RPE cells, thus participates in regulation of cellular autophagy[65-66]. Thioredoxin-1(Trx1) is an anti-inflammatory molecule that binds to thioredoxin-interacting protein (TXNIP) thus prevents free TXNIP to activate NOD-like receptor thermal protein domain associated protein 3 inflammasome in ARPE-19 cells. Under OS, the inhibitory effect is lost with the dissociation of Trx1/TXNIP complex. While Nrf2 activates Trx1 and reduces TXNIP expression as well to alleviate NOD-like receptor thermal protein domain associated protein 3 inflammatory response[67]. Furthermore, Nrf2 deficiency is identified to amplify the imbalance of complement components and complement regulator proteins induced by cigarette smoke extract, thus mediate complement cascade in RPE cells[68]. The additional regulatory role of Nrf2 on mitochondrial function restoration, autophagy promotion and innate immune response further implicates its important factor in the development of AMD.

Complexity of Nrf2 Upstream Regulators and Signaling Pathways The expression and activation of Nrf2 is delicately regulated by other molecules at the transcriptional, translational and post-translational levels. Multiple activated protein kinases such as extracellular signal-regulated kinase kinase kinase 1, transforming growth factor-beta-activated kinase, and apoptosis signal-regulating kinasephosphorylate sites

located within transactivation domain of Nrf2 and effectively induce Nrf2's transcriptional activity[68]. For instance, a recent study pointed that under OS, the 43S pre-initiation complex is attached to Nrf2 mRNA by the far upstream element binding protein 1 protein, increasing the protein's affinity for Nrf2 mRNA[69]. Besides, many non-coding miRNAs are also known to control the activation of Nrf2 by targeting the 3'-untranslated region or other upstream regulators[70]. Considering the complexity of Nrf2 upstream regulation, drugs targeting at other molecules may intentionally influence the expression and activation of Nrf2, and Nrf2 activators can influence other signal pathways partly due to their molecular structures as well. Tertiary butylhydroquinone, an Nrf2 activator, is proved to promote Akt phosphorylation, increase the level of p-Akt S473 protein thus activate phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signal pathway[71]. Another famous Nrf2 activator, sulforaphane can modify many cellular proteins with its highly electrophilic isothiocyanate group[72-73]. Therefore, studying the upstream regulatory mechanism of Nrf2 has an important impact on the future development of drug therapy for AMD, and we will stress it detailedly in the next part.

Regulatory mechanism of Nrf2 expression Under basal conditions, Nrf2 is sealed with Kelch-like erythroid cell-derived protein (Keap1). Two motifs of Keap1, ETGE and DLG, interact with Neh2 domain of Nrf2 to mediate the degeneration of Nrf2[74]. The affinity to Keap1 of ETGE is about 200 times stronger than DLG[75], which determines that the ETGE-mediated interaction is tight and unalterable, therefore the presence or absence of DLG-driven interaction is decisive for the conformational change of Keap1/Nrf2 complex[76]. DLG-driven interaction exists under normal circumstance, Nrf2 is degenerated via the proteasomal pathway to keep its levels low. While under OS, several cysteine residues within Keap1 can be phosphorylated modified, which leads to conformational changes in Keap1[77]. Although Nrf2 is still trapped with Keap1 then, the ubiquitination turns misaligned with Nrf2 lysine residues, and the degeneration of Nrf2 is restrained, thus allows the *de-novo* synthesized Nrf2 to move into the nucleus[77]. After the nuclear translocation of Nrf2, it subsequently heterodimerizes with small Maf proteins and binds to the phase 2 anti-oxidant enzymes (ARE) within a DNA promoter, thus initiates the transcription of anti-oxidant and phase II detoxifying enzymes and neutralizes the increased levels of intracellular ROS[78].

The above processes are intricately regulated within the cell and as shown in Figure 1. Despite Keap1, other molecules including p62 and recombinant cyclin dependent kinase inhibitor 1A (p21) also take part in the regulation of the nuclear translocation of Nrf2 *via* interacting with Keap1 or directly acting on Nrf2. p62 can stabilize Nrf2 and increase the nuclear translocation of Nrf2 by both sequestering Keap1 to autophagic degradation and competing with Nrf2 for Keap1[79-80]. p21, a cyclin dependent kinase, directly interacts with Nrf2 at DLG motifs, and thus competes with Keap1 for Nrf2 binding, and compromise the ubiquitination of Nrf2[81]. Besides, many repressor proteins including BTB and CNC homology 1 (BACH1) can debilitate Nrf2's transcriptional activity by preventing its binding to ARE[82]. Recently, Cai *et al*[46] summarized the drugs which have been shown to treat AMD through the Keap1-Nrf2-ARE pathway, further providing evidences of the regulatory mechanisms via Nrf2 expression.

Noteworthily, glycogen synthase kinase 3β (GSK3β) is a vital negative regulator of Nrf2 that takes part in several steps of Nrf2 activation. Active GSK3β phosphorylates Nrf2 in the Neh6 domain and facilitates the recognition of Nrf2 by β-transducin repeat-containing proteins, an important substrate for the subsequent ubiquitination and proteasomal degradation of Nrf2[83]. Importantly, Nrf2 in the nucleus can still be transported outside of the nucleus. This process can be mediated by nuclear export sequence and is triggered by GSK3β-mediated phosphorylation of Nrf2[84]. Moreover, GSK3β phosphorylates the SRC-related kinase FYN, which subsequently translocated to the nucleus, where it phosphorylates Nrf2 at T568 and promotes the nuclear export and cytoplasmic degradation of Nrf2[85]. Together, these above stress the significant role of GSK3β in downgrading the expression of Nrf2. The activity of GSK3β is precisely regulated by a number of upstream regulators such as active kinases according to the level of cellular OS to maintain cellular redox homeostasis[86]. As GSK3β being suggested to be one of key nodes of pathological changes related to aging and aging-related diseases[87-88], this regulatory network of GSK3β/Nrf2 signaling has also received more attention[89].

All in all, under OS, the anti-oxidant response mediated by upstream factors mainly focuses on promoting cytoplasmic Nrf2 protein abundance, increasing Nrf2 transcriptional activity and inhibiting Nrf2 nuclear exportation. These multiple-step signaling mechanisms may have a synergistic effect, increasing Nrf2 activation in the nucleus with ultrasensitivity[90].

As the upstream regulatory molecules target on the mentioned above key molecules especially GSK3β might be potential effective targets for the treatment of AMD, below we will illustrate the upstream regulatory molecules of Nrf2 reported by existing literatures, and their potential mechanisms and regulatory relationships are shown in Figure 1 and Table 1[91-116].

Pathways that regulate Nrf2 activation

1) AMPK signal pathway Under OS, AMP-activated protein kinase (AMPK) becomes activated as the intracellular

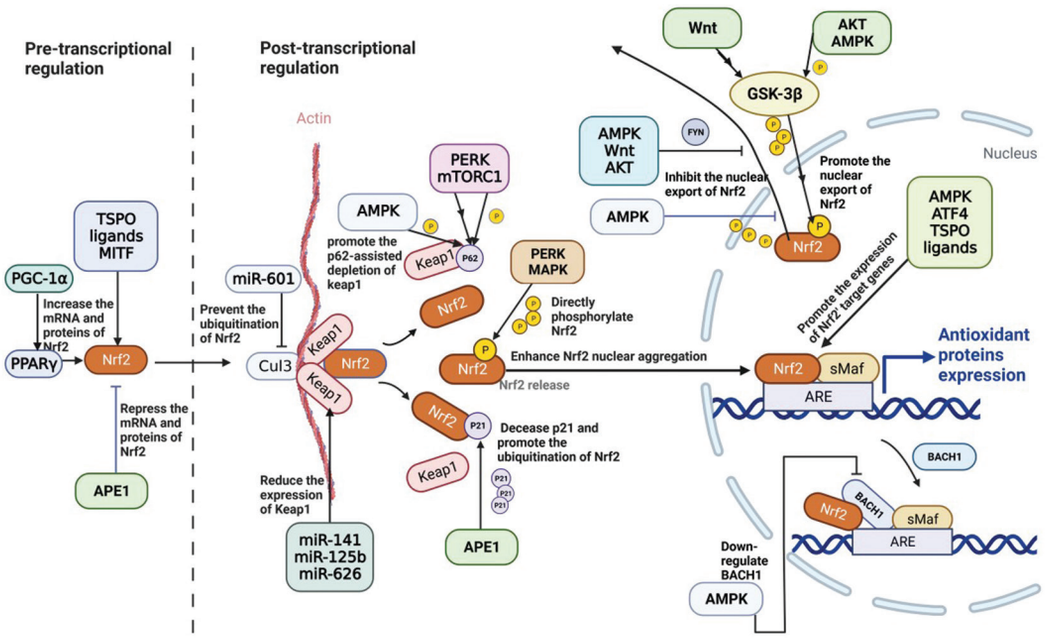

Figure 1 The cellular regulatory mechanism of Nrf2 expression in RPE cells → represents direct activation, →→ represents indirect activation, ⊥ represents inhibition. AMPK: AMP-activated protein kinase; PERK: Protein kinase RNA-like ER kinase; mTORC1: Rapamycin complex 1; p62: Sequestosome-1; APE1: Apyrimidinic endoclease 1; Cul3: Cullin 3; Keap1: Kelch-like ECH-associated protein 1; Nrf2: Nuclear factor erythroid 2-related factor 2; MAPK: Mitogen-activated protein kinase; p21: Cyclin-dependent kinase inhibitor 1A; PPARγ: Peroxisome proliferators-activated receptorsγ; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; MITF: Microphthalmia-related transcription factor; TSPO: Translocator protein; S1R: Sigma-1 receptor; BACH1: BTB and CNC homology 1; AER: Anti-oxidant response element; sMaf: Smooth minor allele frequency; GSK3β: Glycogen synthase kinase-3β; Wnt: Wingless; AKT: Protein kinase B.

Table 1 The cellular upstream regulators of Nrf2 and their acting mechanisms in RPE cells

Regulation sites	Mechanism	Upstream regulators
Regulate on mRNA and protein levels of Nrf2	Repress the mRNA and protein levels of Nrf2	APE1[91]
	Increase the mRNA and protein levels of Nrf2	PGC-1α[92], TSPO ligands[93], MITF[94]
Regulate the depletion of Nrf2 in cytoplasm	Reduce the expression of Keap1	miR-141[95], miR-125b[96], miR-626[97]
	Phosphorylate Nrf2 and change the conformation of Nrf2/Keap1 complex	PERK[98], MAPK[99]
	Promote the p62-assisted depletion of Keap1	AMPK[100], mTORC1[101-102], PERK[103]
	Inhibit βTrCP-triggered degradation of Nrf2 mediated by GSK3β	AMPK[104], Wnt[105], AKT[106]
	Decrease p21 and promote the ubiquitination of Nrf2	APE1[91]
	Inhibit CUL3 to prevent the proteasomal degenerated of Nrf2	miR-601[107]
Regulate the binding of Nrf2 to ARE	Down-regulate BACH1 to inhibit it competing with Nrf2 for ARE sites	AMPK[108]
Regulate the transactivation of Nrf2's target genes	Phosphorylate S374, S408 and S443 in Nrf2	AMPK[109]
	Form bZIP dimers with Nrf2 and promote the expression of HO-1	ATF4[110]
	Unknown	TSPO ligands[111]
Regulate the nuclear export of Nrf2	Phosphorylate Nrf2 in the cytoplasm at S558	AMPK[112]
	Inhibit FYN-mediated nuclear exclusion of Nrf2	AMPK[104], Wnt[113], AKT[106]
Regulate other vital regulators of Nrf2	Phosphorylate and activate AMPK	Sirt1[114], PERK[115]
	Maintain the expression of MAPK kinases	CHOP[116]
	Bind to the promoter region of PGC-1α gene and positively regulate PGC-1α expression	MITF[94]

Nrf2: Nuclear factor erythroid 2-related factor 2; APE1: Apyrimidinic endoclease 1; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; TSPO: Translocator protein; MITF: Microphthalmia-related transcription factor; Keap1: Kelch-like ECH-associated protein 1; PERK: Protein kinase RNA-like ER kinase; MAPK: Mitogen-activated protein kinase; p62: Sequestosome-1; AMPK: AMP-activated protein kinase; mTORC1: Rapamycin complex 1; βTrCP: β-transducin repeat-containing protein; GSK3β: Glycogen synthase kinase-3β; Wnt: Wingless; AKT: Protein kinase B; p21: Cyclin-dependent kinase inhibitor 1A; APE1: Apyrimidinic endoclease 1; CUL3: Cullin 3; BACH1: BTB and CNC homology 1; ARE: Anti-oxidant response element; HO-1: Heme oxygenase 1; ATF4: Activating transcription factor 4; CHOP: C/EBP homologous protein.

energy status is disturbed, and phosphorylates a number of downstream substrates to trigger downstream signaling cascades, thus catalyzes energy metabolism and maintain cellular homeostasis[117-118]. In the retina of mice lacking apolipoprotein E, which is thought to be a useful model of early AMD, the expression of Ataxia-telangiectasia mutated proteins/AMPK pathway is decreased[119]. The activation of AMPK signaling increases the resistance of RPE to the oxidant injury, and reverses the epithelial-mesenchymal transition of RPE and drunsen formation[120-121]. Some pharmacological studies pointed that the effects of AMPK inflict on oxidant stress stem partly from its regulation of Nrf2. Bisphenol A, a commonly used environmental hormone, reduces the phosphorylation of AMPK and suppressed the expression of Nrf2, inducing ROS generation, mitochondrial injury and cytotoxicity in ARPE-19 cells[122]. Conversely, activators of AMPK signaling, including LB-100 and PF-06409577, increase Nrf2 expression and inhibit ultra-violet radiation-induced RPE cells injuries and apoptosis[123].

Activated AMPK can directly phosphorylate Nrf2 in the cytoplasm at S558 to inhibit its nuclear exclusion thus promote its nuclear accumulation[124]. Also, S374, S408, and S443 in Nrf2 are identified to be directly phosphorylated by AMPK, although these sorts of phosphosites seem to be useless for the stabilization and nuclear accumulation of Nrf2, they participate in the transactivation of Nrf2's target genes[112]. Furthermore, Chen *et al*[109] pointed that in RPE cells, AMPK signaling also promotes the coexpression of p62 and Keap1 and boosts p62-assisted depletion of Keap1, leading to enhancement of nuclear Nrf2 expression. This effect has been suggested to be mediated by the phosphorylation of p62 at S294[100]. AMPK also indirectly regulates the expression of Nrf2 through phosphorylating GSK3β at S9 to inhibit βTrCP-triggered degradation of Nrf2 and FYN-mediated nuclear exclusion of Nrf2[125]. Finally, it also down-regulates BACH1 at the mRNA and protein level and inhibits it competing with Nrf2 for ARE sites[104].

Compared with other downstream targeting genes of AMPK, Sirtuin 1 (Sirt1) and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) are focused more in the pathological process of AMD. AMPK phosphorylates PGC-1α and activates Sirt1, which deacetylates PGC-1α and triggers its activation. These three might act as an orchestrated regulatory network for metabolic homeostasis[126]. There are two opposing divergent opinions regarding the effect of deacetylation of Nrf2 by Sirt1 on Nrf2 activity. The earliest study pointed that the acetylation of Nrf2, which mainly happens in its Neh1 domain, enhance binding of Nrf2 to the ARE, while deacetylation conditions caused a decrease in Nrf2-dependent gene transcription through facilitating the accessibility of the relevant nuclear export signal and leading to the relocalization of Nrf2 to the cytoplasmic compartment[127]. But till now, Sirt1's involvement in up-regulating the expression and activation of Nrf2 is thought to be a mechanism for its treatment for AMD. Astragaloside-IV was shown to alleviate the increased contents of ROS and oxidized glutathione and protect ARPE-19 cells from oxidant damage through increasing protein and mRNA levels of Sirt1 and promoting the nuclear localizing of Nrf2[128]. Recent epidemiology study pointed that people who administrate glucosamine orally or parenterally have a lower risk for dry AMD[129], and Han *et al*[130] speculated that the reason might be that this up-regulates the O-GlcNAcylation of Sirt1 at S549 and subsequently amplify its deacetylase effects on Nrf2. The participation of the third factor might explain its anti-oxidant functions and its two opposite effects mentioned above on Nrf2. Despite activating PGC-1α, Sirt1 can also in turn deacetylate and thereby stabilize serine-threonine kinase 11, thus phosphorylate and activate AMPK, which up-regulates Nrf2[131]. Besides, Sirt1 was found to increase the expression of anti-oxidant genes, including manganese-containing SOD, HO-1, and NAD(P)H:quinine oxidoreductase 1 through a forkhead box protein O3-dependent mechanism[132-133].

As an important molecule targeting at mitochondrial biogenesis and anti-OS response, increasing studies have shown that PGC-1α plays a crucial role in the AMPK signaling' mediation of mitochondrial ROS reduction in AMD[134]. Fed *PGC-1α*$^{+/-}$ mice with a high-fat diet to induce OS and the mice with ablated PGC-1α shown elevated ROS levels, increased inflammation and obvious RPE degeneration[135]. In RPE cells exposed to blue light, the expression of the PGC-1α is induced by sulforaphane treatment, resulting activated nuclear translocation of Nrf2 and increased expression of HO-1 gene and production of glutathione[72]. However, the relationship between PGC-1α and Nrf2 might not be unidirectional. Blasiak *et al*[136]. generated the NRF-2/PGC-1α double knockout (dKO) mouse model in which these two genes dominated the occurrence of retinal degradative changes. The RPE cells of dKO mice suffered intense mitochondrial injury, autophagic clearance system impairments and obvious age-dependent RPE degeneration and AMD-like phenotypes, suggesting a potential overlapped and synergistic functions of PGC-1α and Nrf2 in combating the OS in RPE cells. Furthermore, in a model of aging mice obtained by l-buthionine sulfoximine treatment, with continuous decline of glutathione, PGC-1α coactivates Nrf2, adaptively increasing the expression of SOD2 and γ-glutamylcysteine synthetase and buffering the harmful effects of increased SOD2, ROS concentration. While the silencing of PGC-1α by RNAi caused decreased SOD2 and γ-glutamylcysteine synthetase protein contents, also demonstrating regulatory effects of PGC-1α to Nrf2[137].

However, the molecular interaction between Nrf2 and PGC-1α is still unknown. Although it has been acknowledged that PGC-1α can act as a transcriptional coactivator thus activate transcription factors including Nrf2[138], the evidence that can prove the direct binding of Nrf2 to PGC-1α in vitro, even it happens, the exact action sites of PGC-1α on Nrf2 has not been discussed neither. Besides, while St-Pierre *et al*[139] has reported that PGC-1α promoter for binding sites of transcription factors related to OS holds an ARE consensus sequence for the binding of Nrf2, the regulatory function of Nrf2 on the promoter of PGC-1α upon OS has not been observed yet. Together, the interaction between them is probably mediated by a third party factor, and one dominant hypothesis pointed that peroxisome proliferator-activated receptor γ (PPARγ) might be the key factor. Under oxidant stress, PGC-1α acts as transcription factor and activates PPARγ, which subsequently stimulates the transcription of Nrf2[92,140]. Nrf2 can also bind to the promoter region of PPARγ and stimulate its own transcription[141-142]. Whether the complex interaction between Nrf2 and PGC-1α occurs in the RPE cells and the specific mechanism still need more study.

2) Wnt/β-catenin pathway The wingless (Wnt) signaling pathway is a profoundly conserved cell communication pathway that mediates multiple cellular processes like proliferation, differentiation, apoptosis and carcinogenesis[143]. Noteworthily, according to Zhou *et al*[144], stimulation of the canonical Wnt pathway is sufficient to cause OS and dramatically upregulates the expression of vascular endothelial growth factor, nuclear factor κB, and tumor necrosis factor (TNF). Beside, Wnt signaling is also involved in the epithelial-mesenchymal transition of RPE cells, which is a typical pathological manifestation of RPE in AMD[145]. Haines *et al*[146] found that a single-nucleotide polymorphism in lipoprotein receptor-related protein 6, a Wnt receptor, is related with risk for AMD, also indicating the link between dysregulated Wnt signal pathway and AMD. Ebrahimi *et al*[147] pointed that in RPE cells, the interactive decline in Wnt and Nrf2 signaling resulted in dysfunction and apoptosis of RPE and an AMD phenotype. The link between both Wnt pathway and Nrf2 pathway appears to be GSK3β. When facing negative regulators of Wnt signaling, including conditions of OS, inflammation and aging, without Wnt signals, the β-catenin, the key player of Wnt signal, is phosphorylated by GSK3β at the β-catenin destruction complex consisting of GSK3β, Axin1 and activated protein C. Then β-catenin is subsequently degraded, thus the Wnt signal pathway is antagonized[148]. While activated Wnt ligands inactivate GSK3β with phosphorylated dishevelled proteins and stabilize both β-catenin and Nrf2[113]. However, crosstalk between Wnt and Nrf2 pathways has also been reported as impaired Nrf2 signaling causes decreased Wnt activation directly or indirectly. On one hand, Nrf2 reverses repressed Wnt signaling by executing anti-oxidative functions. Shin *et al*[105] demonstrated that exogenous H_2O_2 treatment both decreased the amount of endonuclear β-catenin and the subsequent Tcf/Lef-dependent transcription of its target genes. On the other hand, in ARPE-19 cells, Nrf2 knockdown is found to directly reduce β-catenin, increase p-Gsk3β, and reduce the expression of cyclin D, which is a downstream molecule of Wnt signaling, indicating that Nrf2 influences Wnt signaling[147]. The effects of activated Wnt pathway in RPE cells during the progress of AMD is so complicated that further researches are need before trying to target on the Wnt/Nrf2 signaling.

3) PI3K/Akt/mTOR signal pathway PI3K/Akt signaling also contributes to a number of physiological functions and cellular processes including proliferation, differentiation, survival, metabolism and apoptosis[149]. It has been evidenced to contribute to alleviating OS and relieving induced cell damage[150], these effects are considered to be closely connected with Nrf2, an essential downstream target of PI3K/Akt signaling pathway[151].

In AMD, PI3K/Akt/Nrf2 signaling pathway is activated by H_2O_2 stimulation and ROS accumulation, which has been evidenced to exert cytoprotective functions on RPE cells and modulate oxidative-induced RPE cell death[151]. Piceatannol treatment resulted in Nrf2 signaling activation and increased transcription of anti-oxidant genes in RPE cells, which can be dramatically blocked by PI3K/Akt inhibitor[150]. These results suggest that PI3K-regulated Nrf2 activation plays vital roles in protecting against accumulated oxidative injury in the RPE. However, Chen *et al*[152] pointed that while in PRE cells, PI3K inhibitor can suppress Nrf2 activation induced by 20 μmol/L 4-hydroxy-2-nonenal (HNE), the major lipid peroxidation product in the RPE, it failed to protect PRE cells from cell death induced by higher dose of HNE, suggesting that PI3K/Akt pathway might not be the pathway contributing in RPE cell apoptosis caused by high-dose HNE, and the effects of PI3K/Akt/Nrf2 pathway various due to different levels of OS. Although exist evidence mentioned that Nrf2 nuclear translocation was dynamically balanced with the regulation of PI3K pathway in RPE cells[153], the specific regulatory mechanism still remains unclear. There is no evidence that Akt can directly phosphorylate or activate Nrf2 yet. It is suggested that GSK3β may link up between Akt and Nrf2 as it is phosphorylated at S21 and S9 and inactivated by Akt, and fails to lead to the degradation of Nrf2 and FYN-mediated nuclear exclusion of Nrf2 as we previous mentioned[154].

Mammalian target of rapamycin (mTOR) is a relatively large serine/threonine protein kinase and is one of crucial downstream targets of PI3K/Akt signaling pathway[155].

mTOR is present in two heteromeric protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), among which mTORC1 is involved in regulating transcription and translation, cell cycle, autophagy and is frequently studied in AMD[156-157]. Salvianolic acid A phosphorylated Akt at S6 in APRE19 cells treated with oxidized-low-density lipoprotein (ox-LDL), promoted the Keap1/Nrf2 disassociation, activated Nrf2 and decreased the ROS levels. mTORC1 inhibitor significantly abolished Sal A-induced Nrf2 expression, indicating that Salvianolic acid A's efforts on Nrf2 activation and cytoprotection is mediated by PI3K/Akt/mTORC1 activation in RPE cells[158]. Besides, keratinocyte growth factor is identified to interact with keratinocyte growth factor receptor, thus activate Akt/mTORC1/Nrf2 signaling and counteract ultraviolet-induced RPE cell damage[159]. The activated mTORC1 induces the mTORC1-dependent phosphorylation of p62 at S351 of the Keap1-interacting region, rising the p62's affinity for Keap1 and leading to the sequestration of Keap1 and the stabilization of Nrf2[160-161]. On the other hand, the activation of mTORC1 induces the expression of sestrins[162], the culminated sestrins further interact with p62 and activate the p62-mediated autophagy of Keap1[163]. Then Nrf2 is able to translocate from cytoplasm to nucleus. Note that p62 activation is also crucial for mTORC1 activation, as it in turn interacts with the mTORC1 components Raptor, RagC protein and TNF receptor associated factor 6 in an amino acid-dependent manner, and leads to mTORC1 activation[164]. However, the regulatory relationship between mTORC1 and Nrf2 is not unidirectional, first of all, as we have mentioned before, p62 itself is one of the downstream targets of Nrf2, which forms a regulatory positive feedback loop between mTORC1 and Nrf2[165-166]. This regulatory mechanism has received extensive study under a variety of physiological situations, and it is thought to be a key target for medications to treat neurodegenerative illnesses including Alzheimer's and Parkinson's disorders[167], it might also be useful in the treatment for AMD. Additional research suggested that Nrf2 regulates mTORC1 through interacting with AMPK, study pointed that the knockdown of Nrf2 leaded to the activation of AMPK and consequently to the inhibition of mTOR signaling[168-169]. According to previous studies, AMPK can inactivate the mTORC1 by directly phosphorylating two conserved serine residues on Raptor, a regulatory associated protein of mTOR[170]. Besides, Nrf2 also improves mTOR activity *via* increasing the expression of Ras related GTP binding D, the mTOR activator[171], and directly regulates the mTOR promoter activity[172]. Whether this regulatory mechanism occurs in RPE cells is still an open question.

4) PERK-dependent activation of Nrf2 In PRE cells, the endoplasmic reticulum (ER) safeguards transmembrane and secretory protein synthesis folding thus widely regulates cell stress and apoptosis, maintain cell homeostasis and ensuring RPE cells' critical functions[173], but when RPE cells are under OS, ER stress (ERS) will occur[174], giving rise to a mass of unfolded proteins and subsequent unfolded protein response[175]. The induction of ERS is also identified to promote the activation Nrf2 in RPE cells.

The unfolded protein response mainly contains three ER transmembrane ERS sensors that mediate three corresponding signal pathways: activating transcription factor 6, inositol-requiring enzyme 1 and phosphorylated- protein kinase r-like ER kinase (PERK)[176]. Among them, the PERK pathway is the instant early-response pathway[177], activated PERK phosphorylated eukaryotic initiator factor 2α (eIF2α) and then promotes activating transcription factor 4 (ATF4) translation[178-179]. PERK has been shown to phosphorylate site lying within the amino terminus of Nrf2, trigger a conformational change of Nrf2/Keap1 complex and thus activate Nrf2[98,180]. Besides, another study indicated that in cells affected with hepatic lipotoxicity, PERK also activates the p62-mediated regulation of Keap1-Nrf2 pathway *via* stimulating the phosphorylation of p62 at S351 by AMPK[164] and directly phosphorylating p62[103]. Interestingly, ATF4 is assumed to form classical bZIP dimers with Nrf2 through their leucine zipper structures, and the dimers tied to the SrRE sequence of ho-1 gene to govern the expression of HO-1[110]. Whether these effects occur in RPE cells remains to be investigated. ATF4 also activates the transcription of the C/EBP homologous protein (CHOP), an essential protein for pro-apoptotic process[179,181]. CHOP ablation decreased cell viability in ARPE-19 cells under OS induced by CES[182]. Report suggested that knockdown of CHOP suppresses Nrf2 expression and Nrf2 overexpression also reduces CHOP in RPE cells, which indicated the potential mutual regulation between Nrf2 and CHOP[116]. They also found that knockdown of CHOP decreased phosphorylated p38 mitogen-activated protein kinase[116]. While Nrf2 phosphorylation at S40 by mitogen-activated protein kinases is identified to facilitate Nrf2's dissociation from Keap1 thus promote its nuclear localisation[183], suggesting that CHOP knockdown to some extend down-regulates Nrf2 through MAPK pathway.There have been theories about the methods by which Nrf2 affects CHOP induction, such as: Nrf2 inhibits the binding of other transcription factors to the CHOP promoter by acting as a direct transcriptional repressor and Nrf2 target genes influence CHOP expression directly[180,184].

X-box binding protein 1 (XBP1) is a transcriptional repressor belonging to cAMP response element binding/activating transcription factor basic region-leucine zipper family. Under ERS, inositol-requiring enzyme 1, another ERS sensor, splices XBP1 into spliced XBP1 protein, which then

translocates into the nucleus and regulates the expression of its targeted genes[185]. Recent study shown the XBP1 activation may increase Nrf2 activity through a post-transcriptional mechanism, the overexpression of spliced XBP1 induced obvious increase of Nrf2 protein levels in both nuclear and cytoplasm of RPE cells, while the knockout of XBP1 gene or suppression of XBP1 splicing with pharmacological inhibitor greatly diminishes Nrf2 levels in the RPE[186]. Given the lack of relative studies, the specific mechanisms are still unknown.

Signal molecules regulating Nrf2 activation

1) APE1 Apurinic/apyrimidinic endonuclease 1/Redox Factor-1 (APE1/Ref-1) is known as is the major apyrimidinic endonuclease that highly expressed in choroidal, retinal pericytes and RPE cells[187]. It repairs DNA damage caused by OS, alkylating agents, and ionizing radiation[188]. Besides, the redox function of APE1 reinforces the DNA binding of a number of redox-responsive transcription factors, by which APE1 widely participates in modulating cell stress responses[189]. Although previous work performed in K562 human erythroleukemic cell line implied the role of APE1 in enhancing Nrf2's binding to ARE[190], this positive effects on Nrf2 may not be universal and its role in RPE still need to be revisited. APX3330 (also called E3330) is identified as a small molecule that inhibits APE1 redox function without affecting its DNA repair endonuclease function[191]. Li *et al*[192] pointed that E3330 treatment remarkably decreases the accumulation of ROS and restored the effect of OS on Nrf2 activity induced by ox-LDL in RPE cells, suggesting that the activation of Nrf2 under OS in RPE cells is partially inhibited by APE1. An earlier study carried by Fishel *et al*[91] also demonstrated that the repression of APE1 with E3330 vigorously activates Nrf2 and its downstream targets in a dose-dependent manner in primary pancreatic ductal adenocarcinoma tumor cells. They pointed that APE1 represses Nrf2 expression in mRNA level and also represses the expression of p21 gene. As we have mentioned above, p21 directly interacts with Nrf2 and thus compromises the ubiquitination of Nrf2. These above together may contribute to the downgraded effects of APE1 on Nrf2.

2) S1R/S2R Sigma receptors were classified as Sigma-1 receptor (S1R) and Sigma-2 (S2R) receptor, S1R forms complexes with its binding protein in the plasma membrane, the ER membrane, and the nuclear envelope[193]. The complexes transform from ER membrane to the nuclear membrane and perform the function when interact with its ligands[194]. S2R, also called TMEM97, was only identified recent as an ER resident protein and much less is known about its functional role[195-196]. S1R is highly expressed in distinct types of retina cells including Müller cell, photoreceptor cell[197], retinal ganglion cell[198] and RPE cell[197,199], despite that researches related to S2R are deficient, the expression and prominent functions of S2R in photoreceptor cells and RPE cells have also been reported[197,200]. These together indicate their potential physiological and pathophysiological functions in the progress of retinal diseases. A recent study observed severer oxidative damage in RPE layer under NaIO3 treatment of *S2R* knockout mice, the lack of S2R resulted in diminished abundance of Nrf2 protein and its target gene products, therefore heightened OS and enhanced autophagy[201], indicating that S2R could play a role in alleviating OS by regulating Nrf2 in RPE. However, the definitive mechanism underlying the regulation is still unknown due to lack of research. S1R has been proved to execute anti-oxidant protective effects in other constituent cells of the retina including retinal photoreceptors and Müller cells. S1R is suggested to co-localize with Nrf2 and increase Nrf2-ARE binding, rather than inhibiting Keap1-Nrf2 binding in retinal photoreceptor cell line[202]. While in Müller cells of mice, the absence of S1R resulted in decreased Nrf2 gene and protein expression, as well as increased Keap1 gene and protein expression and an obvious decrease in Nrf2-ARE binding activity in Müller cells of mice[203].The differences suggest the possibility that S1R regulates the activation of Nrf2 in a cell-specific manner. Its effects in RPE will require further studies. Importantly, although both S1R and S2R are observed to positively regulate Nrf2's activity, their effects and underlying mechanisms can not be simply correlated as both the coding genes and functions are different between S1R and S2R.

3) TSPO Translocator protein (TSPO) is thought to be connected with Ca^{2+} homeostasis, steroid synthesis, mitochondrial transition pore regulation, the generation of ROS and the production of energy from nutrients, and the regulation of cholesterol efflux[204]. TSPO is highly expressed in retina component cells, and notably, RPE cells have considerable and constitutive TSPO expression[205], indicating its potential important functions in RPE cells.

Evidences proved the connection between TSPO, Nrf2, and AMD. RPE cells of aging mice showed diminished expression of TSPO and decreased cholesterol efflux, and TSPO ablation resulted in increased absorption and aggregation of ox-LDL, and higher production of ROS[206]. A recent research reported that TSPO ligands protect human RPE cells cultured with reactive microglia supernatants and lysosomal destabilizer by activating Nrf2 signal and thus elevating the expression of its downstream anti-oxidative enzymes. They also showed that three tested TSPO ligands (XBD173, PK11195, and Ro5-4864) display different effects on stressed RPE cells, while all three TSPO ligands significantly increased both mRNA and protein levels of Nrf2 and its downstream genes. PK11195 and Ro5-4864 significantly upregulated the gene and protein levels of HO-1, and XBD173 only increased the expression

of glutaredoxin-1 and thioredoxin-1[93]. The underlying mechanism is still unknown as related study is scarce, but the results to some extent revealed the possibility of activating Nrf2 via regulating TSPO ligands signals as a potential beneficial treatment for AMD.

4) MITF Microphthalmia associated transcription factor (MITF), a member of basic helix-loop-helix leucine zipper transcription factors, is known to play a prominent role in promoting the differentiation of RPE cells and regulating the proliferation of the RPE during development[207]. The MITF gene expression declined in the aging mouse retina compared with young mice, giving rise to dramatically higher ROS levels in RPE and retina[208], while the overexpression of MITF reduced the level of OS in ARPE-19[209]. MITF is found to directly regulate the transcription of Nrf2, promote its nuclear translocation[94] and regulate Nrf2's activity indirectly by *via* binding to the promoter region of *PGC-1α* gene thus positively regulating PGC-1α expression[210]. Besides, as a downstream gene of β-catenin, the transcription of MITF can be influenced by the phosphorylation of GSK3β[211-212], suggesting that any upstream regulators of Nrf2 that mediate GSK3β may also affect Nrf2 *via* MITF signaling. Those findings indicate that MITF regulation of Nrf2 might be related to the oxidative response in RPE and thus influence the progress of AMD, and point the possibility of MITF acting as a potential target for the treatment for AMD.

miRNAs regulation of Nrf2 Both clinical and experimental AMD Studies suggest that miRNAs are closely relevant to AMD pathobiology. miRNAs can influence the activation of Nrf2 and regulate the cellular anti-oxidant response with multiple different mechanisms, which include directly modulating the expression of Nrf2, mediating the nuclear import and export of Nrf2 and indirectly regulating upstream regulators including Keap1 of Nrf2[213]. The potential molecular mechanisms of some upstream miRNAs of Nrf2 found in RPE cells including miRNA-141[214] and miRNA-601[107] have been discussed in cancer studies[215-216]. miRNA-141 is shown to activate Nrf2 by reducing the production of Keap1[95]. miR-601 targets Cullin-3 (CUL3) and mediates the proteasomal degenerated of Nrf2[205]. Here we will discuss some newly found miRNAs upon Nrf2 signaling in RPE cells. CYLD-AS1, a novel oxidation-related lncRNA, sponges miR-134-5p thus indirectly mediates the activity of Nrf2 signaling, protecting RPE cells from OS damage and especially sustaining the normal functions of mitochondria[217]. Xu *et al*[97] found that, either in ARPE-19 cells and primary human RPE cells, miRNA-626 targets on 3'-UTR of Keap1, retardants the subsequent ubiquitination and degeneration of Nrf2, stimulates the stabilization and nuclear translocation of Nrf2 protein, results to increased expression of ARE-dependent genes, including HO-1, NOQ1 and glutamate-cysteine ligase, and consequently protects RPE cells from H_2O_2 stimulation. Importantly, compared with healthy donors, plasma miRNA-626 levels were found obviously downregulated in AMD patients, which indicated that miRNA-626 is not only involved in the regulation of Nrf2 signaling cascade and the pathogenesis of AMD, it has the potential to act as a biomarker for AMD[97]. Likewise, the overexpression of miRNA-125b also inhibits Keap1 expression substantially, thus activates Nrf2 signaling[96], Additionally, miRNA-125b is demonstrated to induce the overexpression of peroxiredoxin-like 2A gene, which is thought to suppress the OS damage *via* regulating Nrf2 signaling in oral squamous cell carcinoma cells[218], whether the similar mechanism also exists in RPE cells is an open question. Besides, Jadeja *et al*[219] reported that overexpression of miRNA-144-3p and -5p participates in the reduced expression of Nrf2 and its downstream anti-oxidant target genes, especially NQO1 and glutamate-cysteine ligase, and promotes the death of cultured human RPE. Collectively, these studies establish miRNA as potential targets for regulating Nrf2 activation, preventing the degeneration of RPE cells and reversing the development of AMD.

Clinical Studies on Nrf2 Upstream Regulators Against AMD Existing clinical researches have explicitly expounded the therapeutic role of the upstream regulators of Nrf2. Metformin, a noted activating agent of AMPK, has been proposed as a candidate drug for AMD by both pre-clinical and clinical studies. In a retrospective study, Jiang *et al*[220] demonstrated that the occurrence of AMD of diabetes mellitus type 2 patients with long-term use of metformin, was significant lower than non-users. In another case-control study including a total of 312 376 control participants, metformin use was also found to be correlated with reduced odds of developing AMD. This association was dose dependent, and low to moderate doses of metformin seemed to be most advantageous[221]. These studies suggest that metformin might be promising for the prevention and treatment of AMD, but as the results from existing researches are still mixed and the effects of metformin has not been quantified accurately yet, further prospective clinical trials are needed in the future.

CONCLUSION

A large number of studies indicate the OS and its subsequent series of pathophysiological changes in RPE cells play an important role in the onset and progress of AMD. As a key transcription factor in regulating antioxidant response and protecting against OS, Nrf2 may represent a potential therapeutic target for preventing AMD. Here we summarize the reported upstream regulatory factors and their possible working mechanisms, and try to provide more possible approaches to activate Nrf2 in addition to existing Nrf2 inducers. However, despite the fact that the upstream

regulators of Nrf2 and their respective regulative mechanisms have been partly demonstrated by previous researches so far, it still has limitations and questions when we focus on it in AMD particularly.

First, there is no perfect animal or cell model can completely mimicked AMD. Although mice have become a popular animal to stimulate AMD, they do not have maculas, which influences the results of experiments to some extent. Besides, ARPE-19 line, a cell line used in a couple of studies, may respond difficultly to the stressors as it does not fully mature and pigment. Second, when evaluating the upstream regulatory factors of Nrf2, most studies have focused on a single upstream signaling or a single molecule. However, the upstream regulatory system of Nrf2 is more like an interactive web. As we have mentioned above, GSK3β plays a vital role in the regulatory work of Nrf2 and it links several essential pathways with Nrf2, but when considering it as a potential target for the treatment for AMD, the complex interaction between these upstream factors should also be seriously concerned. Also, as the regulatory pathways mentioned below are involved in multiple physiological functions with numerous downstream targets, future similar researches need to always keep a watchful eye on the potential side-effects of influencing other essential cellular processes including the response of ER. Beside, although multiple miRNAs were found to directly mediate the activation of Nrf2, we should keep in minds that a single miRNA can modulate a complex network of multiple genes which is an important factor affecting the entry of relevant researches into clinical practice, and further investigations about the complex interactions of miRNAs with their diverse targets are required before inspecting the possibility of miRNAs-based therapeutic strategies in AMD. Thirdly, electrophilic activators of Nrf2 should be cautiously applied in the treatment of AMD as they have off-targets. Their physicochemical property enables them to interact and modify many cellular proteins. For example, sulforaphane is identified to modify hundreds of proteins involved in the regulation of inflammation and OS[212,222], which reminds us also to care about the additional reaction of Nrf2 inducers. Lastly, more experimental and clinical investigations need to be carried out in the future to further confirm the efficiency and safety of Nrf2 activators in AMD. Meanwhile, it deserves notice that the activation of Nrf2 also displays a detrimental side that it may support the progression of severe types of cancers[223].

ACKNOWLEDGEMENTS

Authors' contributions: Writing-original draft preparation Hu ZL, Wang YX, and Lin ZY; Writing-review & editing, Qin Q, Zhao H; Visualisation, Qin Q, Zhao H, Hu ZL, Ren WS, and Liu B. All authors have read and agreed to the published version of the manuscript.

Foundation: Supported by Capital Medical University Scientific Research Grant for Undergraduate Students (No. XSKY2023026).

Conflicts of Interest: Hu ZL, None; **Wang YX,** None; **Lin ZY,** None; **Ren WS,** None; **Liu B,** None; **Zhao H,** None; **Qin Q:** Ophthalmologist licensed in Aixin Clinic of Beijing Shi Jing Shan, and Healthcare Management Center of Peking University People's Hostpital, Beijing, China.

REFERENCES

1 Fleckenstein M, Schmitz-Valckenberg S, Chakravarthy U. Age-related macular degeneration: a review. *JAMA* 2024;331(2):147-157.

2 Wong WL, Su X, Li X, Cheung CM, Klein R, Cheng CY, Wong TY. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health* 2014;2(2):e106-e116.

3 Song P, Du Y, Chan KY, Theodoratou E, Rudan I. The national and subnational prevalence and burden of age-related macular degeneration in China. *J Glob Health* 2017;7(2):020703.

4 Shome I, Thathapudi NC, Aramati BMR, Kowtharapu BS, Jangamreddy JR. Stages, pathogenesis, clinical management and advancements in therapies of age-related macular degeneration. *Int Ophthalmol* 2023;43(10):3891-3909.

5 Shaw PX, Stiles T, Douglas C, Ho D, Fan W, Du H, Xiao X. Oxidative stress, innate immunity, and age-related macular degeneration. *AIMS Mol Sci* 2016;3(2):196-221.

6 Abokyi S, To CH, Lam TT, Tse DY. Central role of oxidative stress in age-related macular degeneration: evidence from a review of the molecular mechanisms and animal models. *Oxid Med Cell Longev* 2020;2020:7901270.

7 Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol* 2001;119(10):1417-1436.

8 Steinberg RH. Interactions between the retinal pigment epithelium and the neural retina. *Doc Ophthalmol* 1985;60(4):327-346.

9 Matsumoto B, Defoe DM, Besharse JC. Membrane turnover in rod photoreceptors: ensheathment and phagocytosis of outer segment distal tips by pseudopodia of the retinal pigment epithelium. *Proc R Soc Lond B Biol Sci* 1987;230(1260):339-354.

10 Shen G, Li Y, Zeng Y, *et al*. Kallistatin deficiency induces the oxidative stress-related epithelial-mesenchymal transition of retinal pigment epithelial cells: a novel protagonist in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2023;64(12):15.

11 AnandBabu K, Sen P, Angayarkanni N. Oxidized LDL, homocysteine, homocysteine thiolactone and advanced glycation end products act as pro-oxidant metabolites inducing cytokine release, macrophage infiltration and pro-angiogenic effect in ARPE-19 cells. *PLoS One* 2019;14(5):e0216899.

12 Cano M, Datta S, Wang L, Liu TY, Flores-Bellver M, Sachdeva M, Sinha D, Handa JT. Nrf2 deficiency decreases NADPH from impaired IDH shuttle and pentose phosphate pathway in retinal pigmented epithelial cells to magnify oxidative stress-induced mitochondrial dysfunction. *Aging Cell* 2021;20(8):e13444.

13 Fan X, Yang Y, Wu G, Kong Y, Zhang Y, Zha X. Circ-CARD6 inhibits oxidative stress-induced apoptosis and autophagy in ARPE-19cells via the miR-29b-3p/PRDX6/PI3K/Akt axis. *Exp Eye Res* 2024;238:109690.

14 Zarbin MA. Age-related macular degeneration: review of pathogenesis. *Eur J Ophthalmol* 1998;8(4):199-206.

15 Si Z, Zheng Y, Zhao J. The role of retinal pigment epithelial cells in age-related macular degeneration: phagocytosis and autophagy. *Biomolecules* 2023;13(6):901.

16 Nakamura S, Noguchi T, Inoue Y, Sakurai S, Nishinaka A, Hida Y, Masuda T, Nakagami Y, Horai N, Tsusaki H, Hara H, Shimazawa M. Nrf2 activator RS9 suppresses pathological ocular angiogenesis and hyperpermeability. *Invest Ophthalmol Vis Sci* 2019;60(6):1943-1952.

17 Li Y, Zhu X, Wang K, Zhu L, Murray M, Zhou F. Ginkgo biloba extracts (GBE) protect human RPE cells from t-BHP-induced oxidative stress and necrosis by activating the Nrf2-mediated antioxidant defence. *J Pharm Pharmacol* 2023;75(1):105-116.

18 Kopacz A, Rojo AI, Patibandla C, Lastra-Martínez D, Piechota-Polanczyk A, Kloska D, Jozkowicz A, Sutherland C, Cuadrado A, Grochot-Przeczek A. Overlooked and valuable facts to know in the NRF2/KEAP1 field. *Free Radic Biol Med* 2022;192:37-49.

19 Brennan LA, Kantorow M. Mitochondrial function and redox control in the aging eye: role of MsrA and other repair systems in cataract and macular degenerations. *Exp Eye Res* 2009;88(2):195-203.

20 Sickel W. Energy metabolism of the retina. *Exp Eye Res* 1969;8(2):252.

21 Abdouh M, Lu M, Chen Y, Goyeneche A, Burnier JV, Burnier MN Jr. Filtering blue light mitigates the deleterious effects induced by the oxidative stress in human retinal pigment epithelial cells. *Exp Eye Res* 2022;217:108978.

22 Yang W, Song C, Gao M, Wang S, Yu H, Li Y. Effects of smoking on the retina of patients with dry age-related macular degeneration by optical coherence tomography angiography. *BMC Ophthalmol* 2022;22(1):315.

23 Rizzolo LJ. Development and role of tight junctions in the retinal pigment epithelium. *Int Rev Cytol* 2007;258:195-234.

24 Wimmers S, Karl MO, Strauss O. Ion channels in the RPE. *Prog Retin Eye Res* 2007;26(3):263-301.

25 Bridges CD, Alvarez RA, Fong SL, Gonzalez-Fernandez F, Lam DM, Liou GI. Visual cycle in the mammalian eye. Retinoid-binding proteins and the distribution of 11-cis retinoids. *Vision Res* 1984;24(11):1581-1594.

26 Markitantova Y, Simirskii V. Endogenous and exogenous regulation of redox homeostasis in retinal pigment epithelium cells: an updated antioxidant perspective. *Int J Mol Sci* 2023;24(13):10776.

27 Lenin RR, Koh YH, Zhang Z, Yeo YZ, Parikh BH, Seah I, Wong W, Su X. Dysfunctional autophagy, proteostasis, and mitochondria as a prelude to age-related macular degeneration. *Int J Mol Sci* 2023;24(10):8763.

28 Barja G. Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. *Antioxid Redox Signal* 2013;19(12):1420-1445.

29 Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, Rayborn ME, Salomon RG, Hollyfield JG. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A* 2002;99(23):14682-14687.

30 Kaarniranta K, Koskela A, Felszeghy S, Kivinen N, Salminen A, Kauppinen A. Fatty acids and oxidized lipoproteins contribute to autophagy and innate immunity responses upon the degeneration of retinal pigment epithelium and development of age-related macular degeneration. *Biochimie* 2019;159:49-54.

31 Krohne TU, Stratmann NK, Kopitz J, Holz FG. Effects of lipid peroxidation products on lipofuscinogenesis and autophagy in human retinal pigment epithelial cells. *Exp Eye Res* 2010;90(3):465-471.

32 Krohne TU, Kaemmerer E, Holz FG, Kopitz J. Lipid peroxidation products reduce lysosomal protease activities in human retinal pigment epithelial cells via two different mechanisms of action. *Exp Eye Res* 2010;90(2):261-266.

33 Koirala D, Beranova-Giorgianni S, Giorgianni F. Early transcriptomic response to OxLDL in human retinal pigment epithelial cells. *Int J Mol Sci* 2020;21(22):8818.

34 Huang P, Liu W, Chen J, Hu Y, Wang Y, Sun J, Feng J. TRIM31 inhibits NLRP3 inflammasome and pyroptosis of retinal pigment epithelial cells through ubiquitination of NLRP3. *Cell Biol Int* 2020;44(11):2213-2219.

35 Ebeling MC, Fisher CR, Kapphahn RJ, Stahl MR, Shen S, Qu J, Montezuma SR, Ferrington DA. Inflammasome activation in retinal pigment epithelium from human donors with age-related macular degeneration. *Cells* 2022;11(13):2075.

36 Marneros AG. Role of inflammasome activation in neovascular age-related macular degeneration. *FEBS J* 2023;290(1):28-36.

37 Macchioni L, Chiasserini D, Mezzasoma L, Davidescu M, Orvietani PL, Fettucciari K, Salviati L, Cellini B, Bellezza I. Crosstalk between long-term sublethal oxidative stress and detrimental inflammation as potential drivers for age-related retinal degeneration. *Antioxidants* 2020;10(1):25.

38 Kim J, Lee YJ, Won JY. Molecular mechanisms of retinal pigment epithelium dysfunction in age-related macular degeneration. *Int J Mol Sci* 2021;22(22):12298.

39 Gelat B, Rathaur P, Malaviya P, Patel B, Trivedi K, Johar K, Gelat R. The intervention of epithelial-mesenchymal transition in homeostasis of human retinal pigment epithelial cells: a review. *J Histotechnol* 2022;45(4):148-160.

40 Anderson DMG, Kotnala A, Migas LG, Patterson NH, Tideman L, Cao DF, Adhikari B, Messinger JD, Ach T, Tortorella S, van de Plas R, Curcio CA, Schey KL. Lysolipids are prominent in subretinal

drusenoid deposits, a high-risk phenotype in age-related macular degeneration. *Front Ophthalmol* 2023;3:1258734.

41 Chong NH, Keonin J, Luthert PJ, Frennesson CI, Weingeist DM, Wolf RL, Mullins RF, Hageman GS. Decreased thickness and integrity of the macular elastic layer of Bruch's membrane correspond to the distribution of lesions associated with age-related macular degeneration. *Am J Pathol* 2005;166(1):241-251.

42 Jhingan M, Singh SR, Samanta A, Arora S, Tucci D, Amarasekera S, Cagini C, Lupidi M, Chhablani J. Drusen ooze: Predictor for progression of dry age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 2021;259(9):2687-2694.

43 Heloterä H, Kaarniranta K. A linkage between angiogenesis and inflammation in neovascular age-related macular degeneration. *Cells* 2022;11(21):3453.

44 Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A* 1994;91(21):9926-9930.

45 Hyttinen JMT, Kannan R, Felszeghy S, Niittykoski M, Salminen A, Kaarniranta K. The regulation of NFE2L2 (NRF2) signalling and epithelial-to-mesenchymal transition in age-related macular degeneration pathology. *Int J Mol Sci* 2019;20(22):5800.

46 Cai ZY, Fu MD, Liu K, Duan XC. Therapeutic effect of Keap1-Nrf2-ARE pathway-related drugs on age-related eye diseases through anti-oxidative stress. *Int J Ophthalmol* 2021;14(8):1260-1273.

47 Maurya M, Bora K, Blomfield AK, Pavlovich MC, Huang S, Liu CH, Chen J. Oxidative stress in retinal pigment epithelium degeneration: from pathogenesis to therapeutic targets in dry age-related macular degeneration. *Neural Regen Res* 2023;18(10):2173-2181.

48 Zhao Z, Chen Y, Wang J, Sternberg P, Freeman ML, Grossniklaus HE, Cai J. Age-related retinopathy in NRF2-deficient mice. *PLoS One* 2011;6(4):e19456.

49 Liu X, Ward K, Xavier C, Jann J, Clark AF, Pang IH, Wu H. The novel triterpenoid RTA 408 protects human retinal pigment epithelial cells against H_2O_2-induced cell injury via NF-E2-related factor 2 (Nrf2) activation. *Redox Biol* 2016;8:98-109.

50 Zhang Y, Yang Y, Yu H, Li M, Hang L, Xu X. Apigenin protects mouse retina against oxidative damage by regulating the Nrf2 pathway and autophagy. *Oxid Med Cell Longev* 2020;2020:9420704.

51 Cui R, Tian L, Lu D, Li H, Cui J. Exendin-4 protects human retinal pigment epithelial cells from H_2O_2-induced oxidative damage via activation of NRF2 signaling. *Ophthalmic Res* 2020;63(4):404-412.

52 Lee JM, Johnson JA. An important role of Nrf2-ARE pathway in the cellular defense mechanism. *BMB Rep* 2004;37(2):139-143.

53 You L, Peng H, Liu J, Cai M, Wu H, Zhang Z, Bai J, Yao Y, Dong X, Yin X, Ni J. Catalpol protects ARPE-19 cells against oxidative stress via activation of the Keap1/Nrf2/ARE pathway. *Cells* 2021;10(10):2635.

54 Clementi ME, Sampaolese B, Sciandra F, Tringali G. Punicalagin protects human retinal pigment epithelium cells from ultraviolet radiation-induced oxidative damage by activating Nrf2/HO-1 signaling pathway and reducing apoptosis. *Antioxidants* 2020;9(6):473.

55 Suh JH, Shenvi SV, Dixon BM, Liu H, Jaiswal AK, Liu RM, Hagen TM. Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proc Natl Acad Sci U S A* 2004;101(10):3381-3386.

56 Zhou J, Chen F, Yan A, Xia X. Madecassoside protects retinal pigment epithelial cells against hydrogen peroxide-induced oxidative stress and apoptosis through the activation of Nrf2/HO-1 pathway. *Biosci Rep* 2020;40(10):BSR20194347.

57 Sachdeva MM, Cano M, Handa JT. Nrf2 signaling is impaired in the aging RPE given an oxidative insult. *Exp Eye Res* 2014;119:111-114.

58 Qi X, Walton DA, Plafker KS, Boulton ME, Plafker SM. Sulforaphane recovers cone function in an Nrf2-dependent manner in middle-aged mice undergoing RPE oxidative stress. *Mol Vis* 2022;28:378-393.

59 Lan L, Wang S, Duan S, Zhou X, Li Y. *Cordyceps militaris* carotenoids protect human retinal endothelial cells against the oxidative injury and apoptosis resulting from H_2O_2. *Evid Based Complement Alternat Med* 2022;2022:1259093.

60 Zhang J, Zhou H, Chen J, Lv X, Liu H. Aloperine protects human retinal pigment epithelial cells against hydrogen peroxide-induced oxidative stress and apoptosis through activation of Nrf2/HO-1 pathway. *J Recept Signal Transduct Res* 2022;42(1):88-94.

61 Xu XR, Yu HT, Yang Y, Hang L, Yang XW, Ding SH. Quercetin phospholipid complex significantly protects against oxidative injury in ARPE-19 cells associated with activation of Nrf2 pathway. *Eur J Pharmacol* 2016;770:1-8.

62 Shen Q, Fang J, Guo H, Su X, Zhu B, Yao X, Wang Y, Cao A, Wang H, Wang L. Astragaloside IV attenuates podocyte apoptosis through ameliorating mitochondrial dysfunction by up-regulated Nrf2-ARE/TFAM signaling in diabetic kidney disease. *Free Radic Biol Med* 2023;203:45-57.

63 Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci U S A* 1994;91(4):1309-1313.

64 Miller DM, Singh IN, Wang JA, Hall ED. Administration of the Nrf2-ARE activators sulforaphane and carnosic acid attenuates 4-hydroxy-2-nonenal-induced mitochondrial dysfunction *ex vivo*. *Free Radic Biol Med* 2013;57:1-9.

65 Saito Y, Yako T, Otsu W, Nakamura S, Inoue Y, Muramatsu A, Nakagami Y, Shimazawa M, Hara H. A triterpenoid Nrf2 activator, RS9, promotes LC3-associated phagocytosis of photoreceptor outer segments in a p62-independent manner. *Free Radic Biol Med* 2020;152:235-247.

66 Wang L, Ebrahimi KB, Chyn M, Cano M, Handa JT. Biology of p62/sequestosome-1 in Age-Related Macular Degeneration (AMD). *Adv Exp Med Biol* 2016;854:17-22.

67 Yang Q, Cai W, Jin H, Shen T, Yu J. Downregulation of inflammatory response via Nrf2/Trx1/TXNIP axis in oxidative stress-induced

ARPE-19 cells and mouse model of AMD. *Oxid Med Cell Longev* 2022;2022:1497813.

68 Wang L, Kondo N, Cano M, Ebrahimi K, Yoshida T, Barnett BP, Biswal S, Handa JT. Nrf2 signaling modulates cigarette smoke-induced complement activation in retinal pigmented epithelial cells. *Free Radic Biol Med* 2014;70:155-166.

69 Dai W, Qu H, Zhang J, Thongkum A, Dinh TN, Kappeler KV, Chen QM. Far Upstream Binding Protein 1 (FUBP1) participates in translational regulation of Nrf2 protein under oxidative stress. *Redox Biol* 2021;41:101906.

70 Padmavathi G, Ramkumar KM. MicroRNA mediated regulation of the major redox homeostasis switch, Nrf2, and its impact on oxidative stress-induced ischemic/reperfusion injury. *Arch Biochem Biophys* 2021;698:108725.

71 Cao Y, Wang J, Wei F, Gu Q, Tian M, Lv HB. Tert-butylhydroquinone protects the retina from oxidative stress in STZ-induced diabetic rats via the PI3K/Akt/eNOS pathway. *Eur J Pharmacol* 2022;935:175297.

72 Yang PM, Cheng KC, Huang JY, Wang SY, Lin YN, Tseng YT, Hsieh CW, Wung BS. Sulforaphane inhibits blue light-induced inflammation and apoptosis by upregulating the SIRT1/PGC-1α/Nrf2 pathway and autophagy in retinal pigment epithelial cells. *Toxicol Appl Pharmacol* 2021;421:115545.

73 Clulow JA, Storck EM, Lanyon-Hogg T, Kalesh KA, Jones LH, Tate EW. Competition-based, quantitative chemical proteomics in breast cancer cells identifies new target profiles for sulforaphane. *Chem Commun* 2017;53(37):5182-5185.

74 Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 1999;13(1):76-86.

75 Tong KI, Katoh Y, Kusunoki H, Itoh K, Tanaka T, Yamamoto M. Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. *Mol Cell Biol* 2006;26(8):2887-2900.

76 Tong KI, Kobayashi A, Katsuoka F, Yamamoto M. Two-site substrate recognition model for the Keap1-Nrf2 system: a hinge and latch mechanism. *Biol Chem* 2006;387(10-11):1311-1320.

77 Kobayashi A, Kang MI, Watai Y, Tong KI, Shibata T, Uchida K, Yamamoto M. Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. *Mol Cell Biol* 2006;26(1):221-229.

78 Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 1997;236(2):313-322.

79 Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, Sou YS, Ueno I, Sakamoto A, Tong KI, Kim M, Nishito Y, Iemura S, Natsume T, Ueno T, Kominami E, Motohashi H, Tanaka K, Yamamoto M. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* 2010;12(3):213-223.

80 Yu M, Wang W, Dang J, Liu B, Xu J, Li J, Liu Y, He L, Ying Y, Cai J, Cheng G, Liu K. Hydrogen sulfide protects retinal pigment epithelium cells against ferroptosis through the AMPK- and p62-dependent non-canonical NRF2-KEAP1 pathway. *Exp Cell Res* 2023;422(1):113436.

81 Chen W, Sun Z, Wang XJ, Jiang T, Huang Z, Fang D, Zhang D. Direct interaction between Nrf2 and p21(Cip1/WAF1) upregulates the Nrf2-mediated antioxidant response. *Mol Cell* 2009;34(6):663-673.

82 Hushpulian DM, Kaidery NA, Dutta D, Sharma SM, Gazaryan I, Thomas B. Emerging small molecule inhibitors of Bach1 as therapeutic agents: Rationale, recent advances, and future perspectives. *Bioessays* 2024;46(1):e2300176.

83 Chowdhry S, Zhang Y, McMahon M, Sutherland C, Cuadrado A, Hayes JD. Nrf2 is controlled by two distinct β-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. *Oncogene* 2013;32(32):3765-3781.

84 Salazar M, Rojo AI, Velasco D, de Sagarra RM, Cuadrado A, Cuadrado A. Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. *J Biol Chem* 2006;281(21):14841-14851.

85 Jain AK, Jaiswal AK. GSK-3beta acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2. *J Biol Chem* 2007;282(22):16502-16510.

86 Liu XY, Lu R, Chen J, Wang J, Qian HM, Chen G, Wu RH, Chi ZL. Suppressor of cytokine signaling 2 regulates retinal pigment epithelium metabolism by enhancing autophagy. *Front Neurosci* 2021;15:738022.

87 Singh A, Kumar T, Velagala VR, Thakre S, Joshi A. The actions of lithium on glaucoma and other senile neurodegenerative diseases through GSK-3 inhibition: a narrative review. *Cureus* 2022;14(8):e28265.

88 Pan HY, Valapala M. Regulation of autophagy by the glycogen synthase kinase-3 (GSK-3) signaling pathway. *Int J Mol Sci* 2022;23(3):1709.

89 Zhi X, Lu H, Ma D, Liu J, Luo L, Wang L, Qin Y. Melatonin protects photoreceptor cells against ferroptosis in dry AMD disorder by inhibiting GSK-3B/Fyn-dependent Nrf2 nuclear translocation. *Biochim Biophys Acta Mol Basis Dis* 2024;1870(2):166969.

90 Liu S, Pi J, Zhang Q. Signal amplification in the KEAP1-NRF2-ARE antioxidant response pathway. *Redox Biol* 2022;54:102389.

91 Fishel ML, Wu X, Devlin CM, *et al*. Apurinic/apyrimidinic endonuclease/redox factor-1 (APE1/Ref-1) redox function negatively regulates NRF2. *J Biol Chem* 2015;290(5):3057-3068.

92 Zhou Y, Peng L, Li Y, Zhao Y. Silent information regulator 1 ameliorates oxidative stress injury via PGC-1α/PPARγ-Nrf2 pathway after ischemic stroke in rat. *Brain Res Bull* 2022;178:37-48.

93 Rashid K, Verhoyen M, Taiwo M, Langmann T. Translocator protein (18 kDa) (TSPO) ligands activate Nrf2 signaling and attenuate

inflammatory responses and oxidative stress in human retinal pigment epithelial cells. *Biochem Biophys Res Commun* 2020;528(2):261-268.

94 Han S, Chen J, Hua J, *et al*. MITF protects against oxidative damage-induced retinal degeneration by regulating the NRF2 pathway in the retinal pigment epithelium. *Redox Biol* 2020;34:101537.

95 Shi L, Wu L, Chen Z, Yang J, Chen X, Yu F, Zheng F, Lin X. MiR-141 activates Nrf2-dependent antioxidant pathway via down-regulating the expression of Keap1 conferring the resistance of hepatocellular carcinoma cells to 5-fluorouracil. *Cell Physiol Biochem* 2015;35(6):2333-2348.

96 Liu JX, Ma DY, Zhi XY, Wang MW, Zhao JY, Qin Y. MiR-125b attenuates retinal pigment epithelium oxidative damage via targeting Nrf2/HIF-1α signal pathway. *Exp Cell Res* 2022;410(1):112955.

97 Xu XZ, Tang Y, Cheng LB, Yao J, Jiang Q, Li KR, Zhen Y. Targeting Keap1 by miR-626 protects retinal pigment epithelium cells from oxidative injury by activating Nrf2 signaling. *Free Radic Biol Med* 2019;143:387-396.

98 Almeida LM, Pinho BR, Duchen MR, Oliveira JMA. The PERKs of mitochondria protection during stress: insights for PERK modulation in neurodegenerative and metabolic diseases. *Biol Rev Camb Philos Soc* 2022;97(5):1737-1748.

99 Shih PH, Yen GC. Differential expressions of antioxidant status in aging rats: the role of transcriptional factor Nrf2 and MAPK signaling pathway. *Biogerontology* 2007;8(2):71-80.

100 Ha S, Jeong SH, Yi K, Chung KM, Hong CJ, Kim SW, Kim EK, Yu SW. Phosphorylation of p62 by AMP-activated protein kinase mediates autophagic cell death in adult hippocampal neural stem cells. *J Biol Chem* 2017;292(33):13795-13808.

101 Bae SH, Sung SH, Oh SY, Lim JM, Lee SK, Park YN, Lee HE, Kang DM, Rhee SG. Sestrins activate Nrf2 by promoting p62-dependent autophagic degradation of Keap1 and prevent oxidative liver damage. *Cell Metab* 2013;17(1):73-84.

102 Duran A, Amanchy R, Linares JF, Joshi J, Abu-Baker S, Porollo A, Hansen M, Moscat J, Diaz-Meco MT. p62 is a key regulator of nutrient sensing in the mTORC1 pathway. *Mol Cell* 2011;44(1):134-146.

103 Lee DH, Park JS, Lee YS, Bae SH. PERK prevents hepatic lipotoxicity by activating the p62-ULK1 axis-mediated noncanonical KEAP1-Nrf2 pathway. *Redox Biol* 2022;50:102235.

104 Fischhuber K, Matzinger M, Heiss EH. AMPK enhances transcription of selected Nrf2 target genes via negative regulation of Bach1. *Front Cell Dev Biol* 2020;8:628.

105 Shin SY, Kim CG, Jho EH, Rho MS, Kim YS, Kim YH, Lee YH. Hydrogen peroxide negatively modulates Wnt signaling through downregulation of beta-catenin. *Cancer Lett* 2004;212(2):225-231.

106 Morgensztern D, McLeod HL. PI3K/Akt/mTOR pathway as a target for cancer therapy. *Anticancer Drugs* 2005;16(8):797-803.

107 Chen ZJ, Rong L, Huang D, Jiang Q. Targeting cullin 3 by miR-601 activates Nrf2 signaling to protect retinal pigment epithelium cells from hydrogen peroxide. *Biochem Biophys Res Commun* 2019;515(4):679-687.

108 Petsouki E, Cabrera SNS, Heiss EH. AMPK and NRF2: interactive players in the same team for cellular homeostasis? *Free Radic Biol Med* 2022;190:75-93.

109 Chen X, Zhu Y, Shi X, Zuo J, Hu T, Wu H, Xia Y, Shi W, Wei W. Ming-mu-di-Huang-pill activates SQSTM1 via AMPK-mediated autophagic KEAP1 degradation and protects RPE cells from oxidative damage. *Oxid Med Cell Longev* 2022;2022:5851315.

110 He CH, Gong P, Hu B, Stewart D, Choi ME, Choi AMK, Alam J. Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. *J Biol Chem* 2001;276(24):20858-20865.

111 Biswas L, Farhan F, Reilly J, Bartholomew C, Shu XH. TSPO ligands promote cholesterol efflux and suppress oxidative stress and inflammation in choroidal endothelial cells. *Int J Mol Sci* 2018;19(12):3740.

112 Matzinger M, Fischhuber K, Pölöske D, Mechtler K, Heiss EH. AMPK leads to phosphorylation of the transcription factor Nrf2, tuning transactivation of selected target genes. *Redox Biol* 2020;29:101393.

113 Li L, Yuan H, Weaver CD, Mao J, Farr GH 3rd, Sussman DJ, Jonkers J, Kimelman D, Wu D. Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1. *EMBO J* 1999;18(15):4233-4240.

114 Zheng Z, Chen H, Li J, Li T, Zheng B, Zheng Y, Jin H, He Y, Gu Q, Xu X. Sirtuin 1-mediated cellular metabolic memory of high glucose via the LKB1/AMPK/ROS pathway and therapeutic effects of metformin. *Diabetes* 2012;61(1):217-228.

115 Zaouali MA, Boncompagni E, Reiter RJ, Bejaoui M, Freitas I, Pantazi E, Folch-Puy E, Abdennebi HB, Garcia-Gil FA, Roselló-Catafau J. AMPK involvement in endoplasmic reticulum stress and autophagy modulation after fatty liver graft preservation: a role for melatonin and trimetazidine cocktail. *J Pineal Res* 2013;55(1):65-78.

116 Huang C, Wang JJ, Ma JH, Jin C, Yu Q, Zhang SX. Activation of the UPR protects against cigarette smoke-induced RPE apoptosis through up-regulation of Nrf2. *J Biol Chem* 2015;290(9):5367-5380.

117 Nanjaiah H, Vallikannan B. Lutein upregulates the PGC-1α, NRF1, and TFAM expression by AMPK activation and downregulates ROS to maintain mtDNA integrity and mitochondrial biogenesis in hyperglycemic ARPE-19 cells and rat retina. *Biotechnol Appl Biochem* 2019;66(6):999-1009.

118 Brown EE, Lewin AS, Ash JD. AMPK may play an important role in the Retinal metabolic ecosystem. *Retinal Degenerative Diseases*. Cham: Springer International Publishing, 2019:477-481.

119 Vessey KA, Jobling AI, Tran MX, Wang AY, Greferath U, Fletcher EL. Treatments targeting autophagy ameliorate the age-related macular degeneration phenotype in mice lacking APOE (apolipoprotein E). *Autophagy* 2022;18(10):2368-2384.

120 Park Y, Jeong Y, Son S, Kim DE. AMPK-induced mitochondrial biogenesis decelerates retinal pigment epithelial cell degeneration under nutrient starvation. *BMB Rep* 2023;56(2):84-89.

121 Kim YK, Hong HK, Yoo HS, Park SP, Park KH. AICAR upregulates ABCA1/ABCG1 expression in the retinal pigment epithelium and reduces Bruch's membrane lipid deposit in ApoE deficient mice. *Exp Eye Res* 2021;213:108854.

122 Chiang YW, Su CH, Sun HY, Chen SP, Chen CJ, Chen WY, Chang CC, Chen CM, Kuan YH. Bisphenol A induced apoptosis via oxidative stress generation involved Nrf2/HO-1 pathway and mitochondrial dependent pathways in human retinal pigment epithelium (ARPE-19) cells. *Environ Toxicol* 2022;37(1):131-141.

123 Li XF, Li SY, Dai CM, Li JC, Huang DR, Wang JY. PP2A inhibition by LB-100 protects retinal pigment epithelium cells from UV radiation via activation of AMPK signaling. *Biochem Biophys Res Commun* 2018;506(1):73-80.

124 Joo MS, Kim WD, Lee KY, Kim JH, Koo JH, Kim SG. AMPK facilitates nuclear accumulation of Nrf2 by phosphorylating at serine 550. *Mol Cell Biol* 2016;36(14):1931-1942.

125 Horike N, Sakoda H, Kushiyama A, Ono H, Fujishiro M, Kamata H, Nishiyama K, Uchijima Y, Kurihara Y, Kurihara H, Asano T. AMP-activated protein kinase activation increases phosphorylation of glycogen synthase kinase 3beta and thereby reduces cAMP-responsive element transcriptional activity and phosphoenolpyruvate carboxykinase C gene expression in the liver. *J Biol Chem* 2008;283(49):33902-33910.

126 Zhang M, Jiang N, Chu Y, Postnikova O, Varghese R, Horvath A, Cheema AK, Golestaneh N. Dysregulated metabolic pathways in age-related macular degeneration. *Sci Rep* 2020;10(1):2464.

127 Kawai Y, Garduño L, Theodore M, Yang J, Arinze IJ. Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization. *J Biol Chem* 2011;286(9):7629-7640.

128 Tang X, Li X, Zhang D, Han W. Astragaloside-IV alleviates high glucose-induced ferroptosis in retinal pigment epithelial cells by disrupting the expression of miR-138-5p/Sirt1/Nrf2. *Bioengineered* 2022;13(4):8240-8254.

129 Feng KM, Chien WC, Chen JT, Chen YH, Chung CH, Sun CA, Chen CL. The impact of glucosamine on age-related macular degeneration in patients: a nationwide, population-based cohort study. *PLoS One* 2021;16(5):e0251925.

130 Han C, Gu Y, Shan H, Mi W, Sun J, Shi M, Zhang X, Lu X, Han F, Gong Q, Yu W. O-GlcNAcylation of SIRT1 enhances its deacetylase activity and promotes cytoprotection under stress. *Nat Commun* 2017;8(1):1491.

131 Lei Y, Lei X, Zhu A, Xie S, Zhang T, Wang C, Song A, Wang X, Shu G, Deng X. Ethanol extract of *Rosa rugosa* ameliorates acetaminophen-induced liver injury via upregulating Sirt1 and subsequent potentiation of LKB1/AMPK/Nrf2 cascade in hepatocytes. *Molecules* 2023;28(21):7307.

132 Zhu C, Dong X, Wang X, Zheng Y, Qiu J, Peng Y, Xu J, Chai Z, Liu C. Multiple roles of SIRT2 in regulating physiological and pathological signal transduction. *Genet Res* 2022;2022:9282484.

133 Yao H, Sundar IK, Ahmad T, Lerner C, Gerloff J, Friedman AE, Phipps RP, Sime PJ, McBurney MW, Guarente L, Rahman I. SIRT1 protects against cigarette smoke-induced lung oxidative stress via a $FOXO_3$ dependent mechanism. *Am J Physiol Lung Cell Mol Physiol* 2014;306(9):L816-L828.

134 Blasiak J, Pawlowska E, Sobczuk A, Szczepanska J, Kaarniranta K. The aging stress response and its implication for AMD pathogenesis. *Int J Mol Sci* 2020;21(22):E8840.

135 Zhang M, Chu Y, Mowery J, Konkel B, Galli S, Theos AC, Golestaneh N. *Pgc-1α* repression and high-fat diet induce age-related macular degeneration-like phenotypes in mice. *Dis Model Mech* 2018;11(9):dmm032698.

136 Blasiak J, Koskela A, Pawlowska E, Liukkonen M, Ruuth J, Toropainen E, Hyttinen JMT, Viiri J, Eriksson JE, Xu H, Chen M, Felszeghy S, Kaarniranta K. Epithelial-mesenchymal transition and senescence in the retinal pigment epithelium of *NFE2L2/PGC-1α* double knock-out mice. *Int J Mol Sci* 2021;22(4):1684.

137 Aquilano K, Baldelli S, Pagliei B, Cannata SM, Rotilio G, Ciriolo MR. p53 orchestrates the PGC-1α-mediated antioxidant response upon mild redox and metabolic imbalance. *Antioxid Redox Signal* 2013;18(4):386-399.

138 Scarpulla RC. Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochim Biophys Acta* 2011;1813(7):1269-1278.

139 St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, Spiegelman BM. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 2006;127(2):397-408.

140 Ikeda Y, Sugawara A, Taniyama Y, Uruno A, Igarashi K, Arima S, Ito S, Takeuchi K. Suppression of rat thromboxane synthase gene transcription by peroxisome proliferator-activated receptor gamma in macrophages via an interaction with NRF2. *J Biol Chem* 2000;275(42):33142-33150.

141 Pi J, Leung L, Xue P, Wang W, Hou Y, Liu D, Yehuda-Shnaidman E, Lee C, Lau J, Kurtz TW, Chan JY. Deficiency in the nuclear factor E2-related factor-2 transcription factor results in impaired adipogenesis and protects against diet-induced obesity. *J Biol Chem* 2010;285(12):9292-9300.

142 Zhong CC, Zhao T, Hogstrand C, Chen F, Song CC, Luo Z. Copper (Cu) induced changes of lipid metabolism through oxidative stress-mediated autophagy and Nrf2/PPARγ pathways. *J Nutr Biochem* 2022;100:108883.

143 Armstrong A, Mandala A, Malhotra M, Gnana-Prakasam JP. Canonical Wnt signaling in the pathology of iron overload-induced oxidative stress and age-related diseases. *Oxid Med Cell Longev* 2022;2022:7163326.

144 Zhou T, Hu Y, Chen Y, Zhou KK, Zhang B, Gao G, Ma J. The pathogenic role of the canonical Wnt pathway in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2010;51(9):4371-4379.

145 Zhou M, Geathers JS, Grillo SL, Weber SR, Wang W, Zhao Y, Sundstrom JM. Role of epithelial-mesenchymal transition in retinal pigment epithelium dysfunction. *Front Cell Dev Biol* 2020;8:501.

146 Haines JL, Schnetz-Boutaud N, Schmidt S, Scott WK, Agarwal A, Postel EA, Olson L, Kenealy SJ, Hauser M, Gilbert JR, Pericak-Vance MA. Functional candidate genes in age-related macular degeneration: significant association with VEGF, VLDLR, and LRP6. *Invest Ophthalmol Vis Sci* 2006;47(1):329-335.

147 Ebrahimi KB, Cano M, Rhee J, Datta S, Wang L, Handa JT. Oxidative stress induces an interactive decline in Wnt and Nrf2 signaling in degenerating retinal pigment epithelium. *Antioxid Redox Signal* 2018;29(4):389-407.

148 Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *EMBO J* 1998;17(5):1371-1384.

149 Wang ZY, Zhang Y, Wu LD, Chen J, Chen ML, Chen CM, Xu QH. Artesunate inhibits proliferation and migration of RPE cells and TGF-β2 mediated epithelial mesenchymal transition by suppressing PI3K/AKT pathway. *Int J Ophthalmol* 2022;15(2):197-204.

150 Hao Y, Liu J, Wang Z, Yu LL, Wang J. Piceatannol protects human retinal pigment epithelial cells against hydrogen peroxide induced oxidative stress and apoptosis through modulating PI3K/akt signaling pathway. *Nutrients* 2019;11(7):1515.

151 Yuan Z, Du W, He X, Zhang D, He W. Tribulus terrestris ameliorates oxidative stress-induced ARPE-19 cell injury through the PI3K/akt-Nrf2 signaling pathway. *Oxid Med Cell Longev* 2020;2020:7962393.

152 Chen J, Wang L, Chen Y, Sternberg P, Cai J. Phosphatidylinositol 3 kinase pathway and 4-hydroxy-2-nonenal-induced oxidative injury in the RPE. *Invest Ophthalmol Vis Sci* 2009;50(2):936-942.

153 Wang L, Chen Y, Sternberg P, Cai J. Essential roles of the PI3 kinase/Akt pathway in regulating Nrf2-dependent antioxidant functions in the RPE. *Invest Ophthalmol Vis Sci* 2008;49(4):1671-1678.

154 Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 1995;378(6559):785-789.

155 Guan R, Kang Z, Li L, Yan X, Gao T. PIK3CA regulates development of diabetes retinopathy through the PI3K/Akt/mTOR pathway. *PLoS One* 2024;19(1):e0295813.

156 Sethna S, Scott PA, Giese APJ, Duncan T, Jian X, Riazuddin S, Randazzo PA, Redmond TM, Bernstein SL, Riazuddin S, Ahmed ZM. CIB2 regulates mTORC1 signaling and is essential for autophagy and visual function. *Nat Commun* 2021;12(1):3906.

157 Madrakhimov SB, Yang JY, Park HY, Park TK. Essential role of mTOR signaling in human retinal pigment epithelial cell regeneration after laser photocoagulation. *Lasers Med Sci* 2019;34(5):1019-1029.

158 Mao K, Shu W, Qiu Q, Gu Q, Wu X. Salvianolic acid A protects retinal pigment epithelium from OX-LDL-induced inflammation in an age-related macular degeneration model. *Discov Med* 2017;23(125):129-147.

159 Hu H, Hao L, Tang C, Zhu Y, Jiang Q, Yao J. Activation of KGFR-Akt-mTOR-Nrf2 signaling protects human retinal pigment epithelium cells from ultra-violet. *Biochem Biophys Res Commun* 2018;495(3):2171-2177.

160 Ichimura Y, Waguri S, Sou YS, *et al*. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. *Mol Cell* 2013;51(5):618-631.

161 Lee B, Kim YH, Lee W, Choi HY, Lee JS, Kim J, Mai DN, Jung SF, Kwak MS, Shin JS. USP13 deubiquitinates p62/SQSTM1 to induce autophagy and Nrf2 release for activating antioxidant response genes. *Free Radic Biol Med* 2023;208:820-832.

162 Fang H, Shi X, Wan J, Zhong X. Role of sestrins in metabolic and aging-related diseases. *Biogerontology* 2024;25(1):9-22.

163 Ala M. Sestrin2 signaling pathway regulates podocyte biology and protects against diabetic nephropathy. *J Diabetes Res* 2023;2023:8776878.

164 Zhang Q, Cai R, Tang G, Zhang W, Pang W. MiR-146a-5p targeting SMAD4 and TRAF6 inhibits adipogenensis through TGF-β and AKT/mTORC1 signal pathways in porcine intramuscular preadipocytes. *J Anim Sci Biotechnol* 2021;12(1):12.

165 Jain A, Lamark T, Sjøttem E, Larsen KB, Awuh JA, Øvervatn A, McMahon M, Hayes JD, Johansen T. p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J Biol Chem* 2010;285(29):22576-22591.

166 Xia Q, Li Y, Xu W, Wu C, Zheng H, Liu L, Dong L. Enhanced liquidity of p62 droplets mediated by Smurf1 links Nrf2 activation and autophagy. *Cell Biosci* 2023;13(1):37.

167 Gureev AP, Sadovnikova IS, Starkov NN, Starkov AA, Popov VN. p62-Nrf2-p62 mitophagy regulatory loop as a target for preventive therapy of neurodegenerative diseases. *Brain Sci* 2020;10(11):847.

168 Jia Y, Wang H, Wang Q, Ding H, Wu H, Pan H. Silencing Nrf2 impairs glioma cell proliferation via AMPK-activated mTOR inhibition. *Biochem Biophys Res Commun* 2016;469(3):665-671.

169 Shen B, Feng H, Cheng J, Li Z, Jin M, Zhao L, Wang Q, Qin H, Liu G. Geniposide alleviates non-alcohol fatty liver disease via regulating Nrf2/AMPK/mTOR signalling pathways. *J Cell Mol Med* 2020;24(9):5097-5108.

170 Xu C, Pan X, Wang D, Guan Y, Yang W, Chen X, Liu Y. O-GlcNAcylation of Raptor transduces glucose signals to mTORC1. *Mol Cell* 2023;83(16):3027-3040.e11.

171 Ji L, Moghal N, Zou X, Fang Y, Hu S, Wang Y, Tsao MS. The NRF2 antagonist ML385 inhibits PI3K-mTOR signaling and growth of lung squamous cell carcinoma cells. *Cancer Med* 2023;12(5):5688-5702.

172 Bendavit G, Aboulkassim T, Hilmi K, Shah S, Batist G. nrf2 transcription factor can directly regulate mtor: linking cytoprotective gene expression to a major metabolic regulator that generates redox activity. *J Biol Chem* 2016;291(49):25476-25488.

173 Zhang SX, Wang JJ, Starr CR, Lee EJ, Park KS, Zhylkibayev A, Medina A, Lin JH, Gorbatyuk M. The endoplasmic reticulum: Homeostasis and crosstalk in retinal health and disease. *Prog Retin Eye Res* 2024;98:101231.

174 Yang YC, Chien Y, Yarmishyn AA, Lim LY, Tsai HY, Kuo WC, Tsai PH, Yang SH, Hong SI, Chen SJ, Hwang DK, Yang YP, Chiou SH. Inhibition of oxidative stress-induced epithelial-mesenchymal transition in retinal pigment epithelial cells of age-related macular degeneration model by suppressing ERK activation. *J Adv Res* 2023:S2090-S1232(23)00167-4.

175 Kroeger H, Chiang WC, Felden J, Nguyen A, Lin JH. ER stress and unfolded protein response in ocular health and disease. *FEBS J* 2019;286(2):399-412.

176 Ding L, Zhu H, Wang K, Huang R, Yu W, Yan B, Zhou B, Wang H, Yang Z, Liu Z, Wang J. Quercetin alleviates cadmium-induced BRL-3A cell apoptosis by inhibiting oxidative stress and the PERK/IRE1α/ATF6 signaling pathway. *Environ Sci Pollut Res Int* 2023;30(60):125790-125805.

177 Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol* 2012;13:89-102.

178 Luo S, Baumeister P, Yang S, Abcouwer SF, Lee AS. Induction of Grp78/BiP by translational block: activation of the Grp78 promoter by ATF4 through and upstream ATF/CRE site independent of the endoplasmic reticulum stress elements. *J Biol Chem* 2003;278(39):37375-37385.

179 Peng W, Wu Y, Peng Z, Qi W, Liu T, Yang B, He D, Liu Y, Wang Y. Cyanidin-3-glucoside improves the barrier function of retinal pigment epithelium cells by attenuating endoplasmic reticulum stress-induced apoptosis. *Food Res Int* 2022;157:111313.

180 Cullinan SB, Diehl JA. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J Biol Chem* 2004;279(19):20108-20117.

181 Averous J, Bruhat A, Jousse C, Carraro V, Thiel G, Fafournoux P. Induction of CHOP expression by amino acid limitation requires both ATF4 expression and ATF2 phosphorylation. *J Biol Chem* 2004;279(7):5288-5297.

182 Cano M, Wang L, Wan J, Barnett BP, Ebrahimi K, Qian J, Handa JT. Oxidative stress induces mitochondrial dysfunction and a protective unfolded protein response in RPE cells. *Free Radic Biol Med* 2014;69:1-14.

183 Huang HC, Nguyen T, Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. *J Biol Chem* 2002;277(45):42769-42774.

184 Sun G, Zhao Z, Lang J, Sun B, Zhao Q. Nrf2 loss of function exacerbates endoplasmic reticulum stress-induced apoptosis in TBI mice. *Neurosci Lett* 2022;770:136400.

185 Shivarudrappa AH, Sharan K, Ponesakki G. Lutein activates downstream signaling pathways of unfolded protein response in hyperglycemic ARPE-19 cells. *Eur J Pharmacol* 2022;914:174663.

186 Chen C, Zhong Y, Wang JJ, Yu Q, Plafker K, Plafker S, Zhang S. Regulation of Nrf2 by X box-binding protein 1 in retinal pigment epithelium. *Front Genet* 2018;9:658.

187 Heisel C, Yousif J, Mijiti M, Charizanis K, Brigell M, Corson TW, Kelley MR. APE1/ref-1 as a novel target for retinal diseases. *J Cell Signal* 2021;2(2):133-138.

188 Kim DV, Diatlova EA, Zharkov TD, Melentyev VS, Yudkina AV, Endutkin AV, Zharkov DO. Back-up base excision DNA repair in human cells deficient in the major AP endonuclease, APE1. *Int J Mol Sci* 2023;25(1):64.

189 Hartman GD, Lambert-Cheatham NA, Kelley MR, Corson TW. Inhibition of APE1/ref-1 for neovascular eye diseases: from biology to therapy. *Int J Mol Sci* 2021;22(19):10279.

190 Iwasaki K, MacKenzie EL, Hailemariam K, Sakamoto K, Tsuji Y. Hemin-mediated regulation of an antioxidant-responsive element of the human ferritin H gene and role of Ref-1 during erythroid differentiation of K562 cells. *Mol Cell Biol* 2006;26(7):2845-2856.

191 Jiang A, Gao H, Kelley MR, Qiao X. Inhibition of APE1/Ref-1 redox activity with APX3330 blocks retinal angiogenesis *in vitro* and *in vivo*. *Vision Res* 2011;51(1):93-100.

192 Li Y, Liu X, Zhou T, Kelley MR, Edwards P, Gao H, Qiao X. Inhibition of APE1/Ref-1 redox activity rescues human retinal pigment epithelial cells from oxidative stress and reduces choroidal neovascularization. *Redox Biol* 2014;2:485-494.

193 Xu Z, Lei Y, Qin H, Zhang S, Li P, Yao K. Sigma-1 receptor in retina: neuroprotective effects and potential mechanisms. *Int J Mol Sci* 2022;23(14):7572.

194 Morales-Lázaro SL, González-Ramírez R, Rosenbaum T. Molecular interplay between the sigma-1 receptor, steroids, and ion channels. *Front Pharmacol* 2019;10:419.

195 Alon A, Schmidt HR, Wood MD, Sahn JJ, Martin SF, Kruse AC. Identification of the gene that codes for the σ_2 receptor. *Proc Natl Acad Sci U S A* 2017;114(27):7160-7165.

196 Sorbi C, Belluti S, Atene CG, Marocchi F, Linciano P, Roy N, Paradiso E, Casarini L, Ronsisvalle S, Zanocco-Marani T, Brasili L, Lanfrancone L, Imbriano C, di Rocco G, Franchini S. BS148 reduces the aggressiveness of metastatic melanoma via sigma-2 receptor targeting. *Int J Mol Sci* 2023;24(11):9684.

197 Mavlyutov TA, Li J, Liu XY, Shen HT, Yang H, McCurdy CR, Pattnaik B, Guo LW. Retinal photoreceptor protection in an AMD-related mouse model by selective sigma-1 or sigma-2 receptor modulation. *Genes* 2022;13(12):2386.

198 Zhao J, Gonsalvez GB, Mysona BA, Smith SB, Bollinger KE. Sigma 1 receptor contributes to astrocyte-mediated retinal ganglion cell protection. *Invest Ophthalmol Vis Sci* 2022;63(2):1.

199 Ola MS, Moore P, El-Sherbeny A, Roon P, Agarwal N, Sarthy VP, Casellas P, Ganapathy V, Smith SB. Expression pattern of sigma receptor 1 mRNA and protein in mammalian retina. *Brain Res Mol Brain Res* 2001;95(1-2):86-95.

200 Bucolo C, Drago F, Lin LR, Reddy VN. Sigma receptor ligands protect human retinal cells against oxidative stress. *Neuroreport* 2006;17(3):287-291.

201 Shen H, Li J, Heisler-Taylor T, Makin R, Yang H, Mavlyutov TA, Gelfand B, Cebulla CM, Guo LW. TMEM97 ablation aggravates oxidant-induced retinal degeneration. *Cell Signal* 2021;86:110078.

202 Wang J, Zhao J, Cui X, Mysona BA, Navneet S, Saul A, Ahuja M, Lambert N, Gazaryan IG, Thomas B, Bollinger KE, Smith SB. The molecular chaperone sigma 1 receptor mediates rescue of retinal cone photoreceptor cells via modulation of NRF2. *Free Radic Biol Med* 2019;134:604-616.

203 Wang J, Shanmugam A, Markand S, Zorrilla E, Ganapathy V, Smith SB. Sigma 1 receptor regulates the oxidative stress response in primary retinal Müller glial cells via NRF2 signaling and system xc (-), the Na(+)-independent glutamate-cystine exchanger. *Free Radic Biol Med* 2015;86:25-36.

204 Yu M, Zhao S. Functional role of translocator protein and its ligands in ocular diseases (Review). *Mol Med Rep* 2024;29(2):33.

205 Klee K, Storti F, Barben M, Samardzija M, Langmann T, Dunaief J, Grimm C. Systemic knockout of Tspo in mice does not affect retinal morphology, function and susceptibility to degeneration. *Exp Eye Res* 2019;188:107816.

206 Farhan F, Almarhoun M, Wong A, Findlay AS, Bartholomew C, Williams MTS, Hurd TW, Shu XH. Deletion of TSPO causes dysregulation of cholesterol metabolism in mouse retina. *Cells* 2021;10(11):3066.

207 Ma X, Li H, Chen Y, Yang J, Chen H, Arnheiter H, Hou L. The transcription factor MITF in RPE function and dysfunction. *Prog Retin Eye Res* 2019;73:100766.

208 Kokkinaki M, Abu-Asab M, Gunawardena N, Ahern G, Javidnia M, Young J, Golestaneh N. Klotho regulates retinal pigment epithelial functions and protects against oxidative stress. *J Neurosci* 2013;33(41):16346-16359.

209 Ma X, Chen H, Jian S, He J, Liu Y, Han S, Chang L, Li P, Chen Y, Liu X, Hu X, Chen Y, Hou L. DAPL1 deficiency in mice impairs antioxidant defenses in the RPE and leads to retinal degeneration with AMD-like features. *Redox Biol* 2023;62:102675.

210 Hua J, Chen H, Chen Y, Zheng G, Li F, Qu J, Ma X, Hou L. MITF acts as an anti-oxidant transcription factor to regulate mitochondrial biogenesis and redox signaling in retinal pigment epithelial cells. *Exp Eye Res* 2018;170:138-147.

211 Widlund HR, Horstmann MA, Price ER, Cui J, Lessnick SL, Wu M, He X, Fisher DE. Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. *J Cell Biol* 2002;158(6):1079-1087.

212 Choi H, Yoon JH, Youn K, Jun M. Decursin prevents melanogenesis by suppressing MITF expression through the regulation of PKA/CREB, MAPKs, and PI3K/Akt/GSK-3β cascades. *Biomed Pharmacother* 2022;147:112651.

213 Ashrafizadeh M, Ahmadi Z, Samarghandian S, Mohammadinejad R, Yaribeygi H, Sathyapalan T, Sahebkar A. MicroRNA-mediated regulation of Nrf2 signaling pathway: Implications in disease therapy and protection against oxidative stress. *Life Sci* 2020;244:117329.

214 Tang CZ, Li KR, Yu Q, Jiang Q, Yao J, Cao C. Activation of Nrf2 by Ginsenoside Rh3 protects retinal pigment epithelium cells and retinal ganglion cells from UV. *Free Radic Biol Med* 2018;117:238-246.

215 Fabrizio FP, Sparaneo A, Muscarella LA. NRF2 regulation by noncoding RNAs in cancers: the present knowledge and the way forward. *Cancers* 2020;12(12):3621.

216 Cuzziol CI, Castanhole-Nunes MMU, Pavarino ÉC, Goloni-Bertollo EM. MicroRNAs as regulators of VEGFA and NFE2L2 in cancer. *Gene* 2020;759:144994.

217 Du H, Huang Z, Zhou X, *et al*. Oxidative stress-induced lncRNA CYLD-AS1 promotes RPE inflammation via Nrf2/miR-134-5p/NF-κB signaling pathway. *FASEB J* 2022;36(10):e22577.

218 Chen YF, Wei YY, Yang CC, Liu CJ, Yeh LY, Chou CH, Chang KW, Lin SC. MiR-125b suppresses oral oncogenicity by targeting the anti-oxidative gene PRXL2A. *Redox Biol* 2019;22:101140.

219 Jadeja RN, Jones MA, Abdelrahman AA, Powell FL, Thounaojam MC, Gutsaeva D, Bartoli M, Martin PM. Inhibiting microRNA-144 potentiates Nrf2-dependent antioxidant signaling in RPE and protects against oxidative stress-induced outer retinal degeneration. *Redox Biol* 2020;28:101336.

220 Jiang J, Chen Y, Zhang H, Yuan W, Zhao T, Wang N, Fan G, Zheng D, Wang Z. Association between metformin use and the risk of age-related macular degeneration in patients with type 2 diabetes: a retrospective study. *BMJ Open* 2022;12(4):e054420.

221 Blitzer AL, Ham SA, Colby KA, Skondra D. Association of metformin use with age-related macular degeneration: a case-control study. *JAMA Ophthalmol* 2021;139(3):302-309.

222 Ruhee RT, Suzuki K. The integrative role of sulforaphane in preventing inflammation, oxidative stress and fatigue: a review of a potential protective phytochemical. *Antioxidants* (*Basel*) 2020;9(6):E521.

223 Ngo HKC, Le H, Surh YJ. Nrf2, A target for precision oncology in cancer prognosis and treatment. *J Cancer Prev* 2023;28(4):131-142.