**Sulforaphane ameliorates amyloid-β-induced inflammatory injury by suppressing the PARP1/SIRT1 pathway in retinal pigment epithelial cells**

Song Wang and Yu-jie Tang

Department of Pharmacy, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang, Hubei, China

**ABSTRACT**

Age-associated macular degeneration (AMD) is a progressive eye disorder that leads to irreversible impairment of central vision, and effective therapies are lacking. Here, we explore how oligomeric amyloid-β1-42 can trigger inflammatory injury in retinal pigment epithelial cells and how sulforaphane can mitigate such injury. ARPE-19 retinal pigment epithelial cells expressing low, endogenous, or high levels of poly(ADP-ribose) polymerase (PARP1) were treated with oligomeric amyloid-β1-42 in the presence or absence of various signaling inhibitors or sulforaphane. Cell viability, apoptosis, inflammatory responses, and activity of the PARP1/Sirtuin (SIRT1) axis were assayed. Treating ARPE-19 cells with oligomeric amyloid-β1-42 promoted the production of IL-1β, IL-6, IL-8, and TNF-α, which was partially reversed by inhibiting PARP1 and activating SIRT1. PARP1 was found to act upstream of SIRT1, and expression of the two proteins correlated negatively with each other. Sulforaphane also mitigated the injury due to oligomeric amyloid-β1-42 through a mechanism involving inactivation of the PARP1/SIRT1 pathway. Oligomeric amyloid-β1-42 can trigger AMD-like injury in retinal pigment epithelium by activating PARP1 and repressing SIRT1. Moreover, sulforaphane can induce cell viability and SIRT1 expression, but reduce cell apoptosis, the activity of caspase-3 or -9, and PARP1 expression in oAβ1-42-treated cells. However, PARP1 inactivation or SIRT1 activation weaken these effects. In summary, sulforaphane reduces the inflammatory injury induced by oAβ1-42 in ARPE-19 cell by inactivating the PARP1/SIRT1 pathway. Thus, the compound may be an effective therapy against AMD.

**CONTACT** Yu-jie Tang ty475700560@163.com Department of Pharmacy, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, 136 Jingzhou Road, Xiangyang, China

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Introduction**

Age-associated macular degeneration (AMD) leads to vision loss among elderly and the prevalence of AMD approximate 24.8% [1]. Degeneration at the ocular interface between the retina and the underlying choroid causes the progressive loss of central vision [2]. Several risk factors for AMD progression have been identified, especially arteriosclerosis, obesity, and smoking [3]. Retinal pigment epithelial (RPE) cells, located closely to the choroidal capillaries, drive AMD because they are particularly vulnerable to inflammatory responses [4]. Normally, RPE cells maintain homeostasis of the outer retina by absorbing light, swallowing old rod outer segments, transporting nutrients or ions, protecting against oxidative stress, and secreting inflammatory cytokines [5,6]. Drusen in AMD patients contains aggregates of amyloid beta (Aβ), the monomer of which contains 39–43 amino acids, and the same aggregates are found in amyloid plaques in patients with Alzheimer’s disease (AD) [7,8]. Such aggregates in drusen are associated with elevated secretion of inflammatory cytokines from RPE cells [9].

Exposing cultures of RPE cells to oligomeric Aβ1-42 has proven to be a good in vitro model of AMD: the oligomers stimulate oxidative stress, apoptosis, and secretion of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) [10–12]. These cytokines are also elevated in patients with AMD [13,14]. Thus, exploring drugs that can repress the inflammatory response in RPE cells may be effective treatments against AMD. Moreover, poly (ADP-ribose) polymerase (PARP1) functions as a upstream molecule of SIRT1, which regulates the inflammatory response to inducing the upregulation of NF-κB, IL-1, and TNF-α [15]. However, whether the PARP1/SIRT1 axis is
involved in the inflammatory injury induced by αAβ_{1-42} in RPE cells remains unclear.

The isothiocyanate sulforaphane, present predominantly in several cruciferous vegetables, such as conventional Chinese cabbage and broccoli. It exerts anti-inflammatory effects [16], and it can ameliorate injury to RPE cells caused by white light and inflammation caused by blue light [17,18]. How sulforaphane exerts these effects to modulate the signal transduction of the PARP1/SIRT1 pathway is unclear. Thus, the present study explored how oligomeric Aβ_{1-42} can induce inflammatory injury in RPE cells and how sulforaphane can reverse such damage.

Here, we supposed that sulforaphane could decrease the inflammatory injury in αAβ_{1-42}-treated cells by inhibiting the PARP1/SIRT1 signaling pathway. To begin to identify its anti-inflammatory mechanisms of action, the present study examined the effects of sulforaphane on the cell viability, apoptosis, and inflammatory response in αAβ_{1-42}-treated ARPE-19 cells, as well as the potential role of PARP1/SIRT1 in mediating those effects.

Materials and methods

Chemicals and reagents

Sulforaphane, oligomeric Aβ_{1-42}, the SIRT1 agonist SRT1720, and the SIRT1 inhibitor nicotinamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lipofectamine 2000 was provided by Sigma-Aldrich. The antibodies against cleaved caspase-3 (catalog no. #ab32043), cleaved caspase-3 (ab2324), PARP1 (ab32064), SIRT1 (ab189494), and β-actin (ab7817) were obtained from Abcam (UK).

Cell culture and treatment

ARPE-19 cells were obtained from the American Type Tissue Culture Collection and cultured in Dulbecco’s modified Eagle’s medium containing the F-12 nutrient mixture (DMEM/F12) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. Cultures were maintained at 37 °C in an atmosphere of 5% CO₂ and 95% air. All cell culture reagents were purchased from Gibco (Grand Island, MA, USA).

Cultures in the logarithmic phase were used in the experiments described below. Depending on the experiment, cells were treated with the following reagents (Sigma-Aldrich, St. Louis, MO, USA): sulforaphane (1–50 μM), oligomeric Aβ_{1-42} (0.1–10 μM), the SIRT1 agonist SRT1720 (0.1, 1, and 5 μM; Sigma-Aldrich), or the SIRT1 inhibitor nicotinamide (2 μM; Sigma-Aldrich). The comprehensive flow sheet abstract was shown in Graphical Abstract.

Under- and overexpression of PARP1 in RPE cells

Human PARP1 cDNA was cloned into pDNA3.1 (Invitrogen, Shanghai, China) to generate a plasmid over-expressing PARP1. A short interfering RNA (siRNA) targeting PARP1 (5’-TATCAACCAGGCTACGTGA-3’) and a negative control siRNA were generated by GenePharm (Shanghai, China). ARPE-19 cells were transfected with the overexpression plasmid or with siRNAs using Lipofectamine 2000 (Sigma-Aldrich) according to the manufacturer’s directions. Under- and overexpression of PARP1 were confirmed by western blot at 24 h after transfection.

Cell counting kit-8 (CCK-8) assay

ARPE-19 cells were seeded at 1 × 10^{4} cells/well in 96-well plates and cultured overnight. After the indicated transfection or treatment, cell viability was measured using the CCK-8 assay (Invitrogen). In brief, the cells were washed twice with phosphate-buffered saline (PBS; Invitrogen); then, 10 μL of CCK-8 solution was added to each well, and the plates were incubated for 2 h at 37 °C in an atmosphere of 5% CO₂ and 95% air. Optical density was measured at 450 nm using a microplate reader (Axiovert S100, Zeiss, Germany).

Flow cytometry of apoptosis

Two-color flow cytometry was performed to measure apoptosis of ARPE-19 cells after the indicated treatments. Briefly, cells were harvested by trypsinization, rinsed, resuspended in DMEM/F12 medium supplemented with 2% FBS, and incubated at
37 °C for 90 min with 5 µg/mL of Annexin V-FITC (Sigma-Aldrich). Next, the cells were counterstained with 2 µg/mL propidium iodide (Sigma-Aldrich) at room temperature for 5 min and analyzed by flow cytometry (BD Biosciences, San Jose, CA, USA). Double-stained cells were considered apoptotic [18].

**Caspase-3 and –9 activity**

The activity of caspase-3 and –9 in ARPE-19 cells was detected using commercial kits (Sigma-Aldrich). The treated cells were suspended in a lysis buffer and incubated on ice for 12 min. The cell homogenates were centrifuged at 14,000 g for 10 min at 4 °C, and the supernatants were incubated with the kit reagent at 37 °C for 2 h. Absorbance was measured at 405 nm.

**Enzyme-linked immunosorbent assay (ELISA)**

Levels of IL-1β, IL-6, IL-8, and TNF-α secreted by ARPE-19 cells into the culture medium were measured using commercial ELISA kits (eBioscience, San Diego, CA, USA) according to the manufacturer’s directions. Optical density was measured at 450 nm using a microplate reader (Axiovert S100, Zeiss, Germany), and the measurements were converted to concentrations using a standard curve.

**Western blot analysis**

Total proteins were extracted from treated cells using a radioimmununoprecipitation assay (RIPA) buffer (Invitrogen) for 30 min on ice. The suspension was centrifuged at 14,000 g for 10 min at 4 °C; then, the total protein concentration in the supernatant was estimated using the BCA assay. Equal amounts of protein from different cultures were denatured by boiling at 100 °C for 5 min; then, 50 µg was separated on a Tris-Tricine gel at 70 V for 3 h. Fractionated proteins were transferred onto a PVDF membrane at 120 mA for 2 h, which was blocked using 5% nonfat milk in TBS-Tween. Membranes were incubated overnight at 4 °C with primary antibodies (all diluted 1:1000; Abcam, UK) against cleaved caspase-3 (catalog no. #ab32043), cleaved caspase-3 (ab2324), PARP1 (ab32064), SIRT1 (ab189494) and β-actin (ab7817). Next, membranes were incubated at room temperature for 1.5 h with horseradish peroxidase-conjugated goat anti-rabbit antibody (diluted 1:3000; Abcam). Finally, images were photographed using the Tanon ECL detection system (Bio-Tanon, Shanghai, China) and quantified using Image J software [18].

**Statistical analysis**

All results were reported as mean ± SD. Differences were assessed for significance using a two-tailed Mann–Whitney test and a one-way ANOVA [18]. Differences associated with P < 0.05 were considered significantly. All graphs were plotted using GraphPad Prism 8.0 software (San Diego, CA, USA).

**Results**

In our study, we supposed that sulforaphan could reduce the inflammatory injury in oAβ1-42-treated cells by repressing the PARP1/SIRT1 signaling pathway. To confirm the anti-inflammation effects of sulforaphane, we first observed its effects on the cell viability, apoptosis, and inflammatory response in oAβ1-42-treated ARPE-19 cells. Then, the potential roles of PARP1/SIRT1 signal transduction in oAβ1-42-treated ARPE-19 cells were explored.

**Oligomeric Aβ1-42 induces inflammatory injury in ARPE-19 cells by suppressing SIRT1**

Aβ peptide was incubated at concentrations of 0.1–10 µM at 37 °C for 24 h, and the resulting oligomers were found to sharply reduce the viability of ARPE-19 cells at concentrations exceeding 1 µM (Figure 1a). Since a concentration of 10 µM reduced viability by more than 50%, we selected this concentration for subsequent experiments.

Oligomeric Aβ1-42 substantially reduced the viability of ARPE-19 cells, and this was associated with the upregulation of cleaved-caspase-3 and –9, an increase in the activity of caspase-3 and –9, increased apoptosis, and elevated production of the pro-inflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α Figure 1(b-h). These effects of oligomeric Aβ1-42 were antagonized by the SIRT1
agonist SRT1720 in a dose-dependent manner. These findings suggest that oligomeric Aβ₁₋₄₂ may promote inflammatory injury in ARPE-19 cells by inhibiting SIRT1.

**PARP1 helps mediate inflammatory injury induced in ARPE-19 cells by oligomeric Aβ₁₋₄₂**

Since PARP1 has been linked to the pathogenesis in AMD, we explored whether altering PARP1 expression influenced the injury induced in ARPE-19 cells by oligomeric Aβ₁₋₄₂. First, we knocked down PARP1 expression in cells by transfecting them with an anti-PARP1 siRNA (Figure 2a). Then, we compared the effects of oligomeric Aβ₁₋₄₂ treatment in the presence or absence of such knockdown. We found that PARP1 down-regulation partially reversed the injury (Figure 2b-h), suggesting that oligomeric Aβ₁₋₄₂ promotes inflammatory injury in ARPE-19 cells by activating PARP1 and suppressing SIRT1 expression.
We further tested this conclusion by evaluating the effects of oligomeric $\text{A}\beta_{1-42}$ on the inflammatory injury in ARPE-19 cells overexpressing PARP1. We further evaluated these effects in the presence or absence of the SIRT1 agonist SRT1720. First, we confirmed that we were able to drive PARP1 overexpression from a transfected plasmid (Figure 3a). Then, we found that PARP1 overexpression reduced the cell viability and increased apoptosis, activity of caspase-3 and $-9$, as well as levels of IL-1$\beta$, IL-6, IL-8 and TNF-$\alpha$ in the culture medium (Figure 3b). All these effects of PARP1 overexpression were partially reversed by SRT1720. These results suggest that PARP1 serves as an upstream regulator of SIRT1.

**Sulforaphane promotes viability of ARPE-19 cells exposed to oligomeric $\text{A}\beta_{1-42}$**

Treating ARPE-19 cells with sulforaphane at concentrations below 20 $\mu$M did not reduce their viability, whereas concentrations of 50 $\mu$M strongly reduced viability (Figure 4a). Therefore, subsequent experiments were carried out using sulforaphane at 5–20 $\mu$M. Within this concentration range, sulforaphane partially reversed the effects of oligomeric $\text{A}\beta_{1-42}$ on the viability, apoptosis, and activity of caspase-3 and $-9$ in a dose-dependent manner (Figure 4b-e).

**Sulforaphane mitigates inflammatory injury induced by oligomeric $\text{A}\beta_{1-42}$ in ARPE-19 cells**

Next, we examined whether sulforaphane exerts its protective effects by reducing the inflammatory response induced by oligomeric $\text{A}\beta_{1-42}$. Indeed, we found that treating cells with oligomeric $\text{A}\beta_{1-42}$ strongly induced the production of IL-1$\beta$, IL-6, IL-8, and TNF-$\alpha$, which sulforaphane reversed in a dose-dependent way (Figure 5a-d). According to the results, 20 $\mu$M of sulforaphane showed the best effect, and it might be involved in the inhibitory effect of sulforaphane on NF-κB signaling, which could be activated by SIRT1 [15].

**Sulforaphane exerts anti-inflammatory effects by repressing the PARP1/SIRT1 pathway**

To explore how sulforaphane protects ARPE-19 cells against inflammatory injury induced by...
Figure 4. Sulforaphane protected ARPE-19 cells from death induced by oligomeric Aβ_{1-42} (oAβ_{1-42}). (a-b) Viability after 24-h exposure to (a) 1–50 μM sulforaphane alone, or (b) 10 μM oAβ_{1-42} and the indicated concentrations of sulforaphane (SFN). (c) Apoptosis after 24-h exposure to 10 μM oAβ_{1-42} and the indicated concentrations of SFN. (d-e) Activity of caspase-3 and −9 after 24-h exposure to 10 μM oAβ_{1-42} and the indicated concentrations of SFN. *P < 0.05, compared to control group (CN); #P < 0.05, compared to oAβ_{1-42} group.

Figure 5. Sulforaphane’s protective effects involve mitigation of the inflammatory response induced by oligomeric Aβ_{1-42} (oAβ_{1-42}). Cells were treated for 24 h with 10 μM oAβ_{1-42} and different concentrations of sulforaphane (SFN). Levels of (a) IL-1β, (b) IL-6, (c) IL-8, and (d) TNF-α were assayed in the culture medium. *P < 0.05, compared to control group (CN); #P < 0.05, compared to oAβ_{1-42} group.
oligomeric Aβ1-42, we examined the compound’s effects on the expression of PARP1 and SIRT1. Sulforaphane reversed the Aβ1-42-induced down-regulation of SIRT1 and upregulation of PARP1 in a dose-dependent manner Figure 6(a-d). These effects of sulforaphane were antagonized by PARP1 overexpression (Figure 7) or pretreatment with the SIRT1 inhibitor nicotinamide (Figure 8). These results suggest that sulforaphane protects against Aβ1-42-induced inflammatory injury by inhibiting PARP1 and upregulating SIRT1.

**Discussion**

Here, we show that sulforaphane can mitigate the cell death and inflammatory responses induced by oligomeric Aβ1-42 in RPE cells. We demonstrate that it exerts these effects by inhibiting PARP1/SIRT1 signaling. Thus, sulforaphane may be a promising candidate drug to treat AMD [19]. Sulforaphane, therefore, shows promising treatment efficacy in a system that models many aspects of AMD [20,21]. We performed our experiments with 10 μM oligomeric Aβ1-42 because this concentration strongly reduced viability. Our results should be verified and extended in studies examining other concentrations, since the concentration can alter the RPE cell structure and function without killing the cells [22].

PARP1 catalyzes the transfer of ADP-ribose to target proteins via NAD [23,24]. In response to minor DNA damage, PARP1 plays a protective function by recruiting repair proteins to the DNA [25]. On the other hand, extensive DNA damage can overactivate PARP1, leading to an energy crisis and parthanatos, a type of necrosis [26]. SIRTs, which like PARPs rely on NAD+ for their activity [27], are associated with metabolism, cell growth, inflammatory response, and autophagy [28]. In particular, SIRT1 modulates inflammation by altering the expression of nuclear factor kappa B (NF-κB), pancreatic and duodenal homeobox factor 1 (PDX1), IL-1, forkhead box class O (FOXO), and TNF-α [29,30].
SIRT1 has been implicated in various disorders, including retinal degenerative diseases [31,32].

We found evidence that PARP1 functions upstream of SIRT1 in AMD: in our experiments in ARPE-19 cells exposed to oligomeric Aβ1-42, PARP1 promoted inflammation and SIRT1 activation, whereas SIRT1 inhibited inflammatory response. PARP1 has been shown to repress SIRT1 activity in other disorders associated with muscles and neurons [33]. In contrast, SIRT1 has been shown to inhibit PARP1 activity and thereby protect cardiomyocytes under ischemic stress [34]. It is noteworthy that SIRT1 invariably promotes cell proliferation, while PARP1 can promote or inhibit it, depending on the physiological context.

PARP1 and SIRT1 mutually regulate the inflammatory response: in various diseases, PARP1 promotes the production of inflammatory cytokines, whereas SIRT1 represses it [35]. Thus, manipulating the PARP1/SIRT1 pathway may be a strategy to treat inflammation-associated disorders. Our experiments suggest that this is also true in AMD: PARP1 underexpression and the SIRT1 agonist SRT1720 reduced inflammatory injury in ARPE-19 cells induced by oligomeric Aβ1-42, whereas PARP1 overexpression and the SIRT1 inhibitor nicotinamide caused the opposite effects. Our results suggest that PARP1-mediated SIRT1 activation may treat AMD, which highlights the need to identify specific inhibitors of the PARP1/SIRT1 pathway.

Our results extend the range of cell types and contexts in which sulforaphane has been shown to reduce PARP1 activity and induce SIRT1 expression to protect from cell injury [36,37]. Future studies should explore in detail how sulforaphane regulates PARP1 and SIRT1. In addition, sulforaphane has been demonstrated to enhance the ability of RPE cells against oxidative stress, but the detailed molecular mechanisms were not studied [38–40]. Moreover, our study has shown that sulforaphane could repress PARP1 and induce SIRT1 expression to reduce the inflammatory injury of RPE cells.
Oligomeric Aβ<sub>1-42</sub> activates an inflammatory response, promotes apoptosis, and reduces viability in RPE cells, which may contribute to AMD. Sulforaphane mitigates all these effects, and it appears to do so by inhibiting the PARP1/SIRT1 axis. The findings of the current investigation provide insights into how sulforaphane may be a useful treatment against retinal inflammatory injury and AMD.

**Limitations and future direction of this study**

However, this study included several limitations. First, we did not overexpressed SIRT1 in ARPE19 cells to assess its effect on the ability of sulforaphane to decrease inflammatory injury of Aβ<sub>1-42</sub>-treated cells. Next, more detection methods should be used to detect cell apoptosis. Finally, the *in vivo* experiments should be performed to confirm our results.

**Highlights**

(1) Oligomeric amyloid-β<sub>1-42</sub> promotes inflammatory injury in ARPE-19 cells.

(2) PARP1/SIRT1 axis activation induces inflammatory injury in ARPE-19 cells.

(3) Sulforaphane reduces inflammatory injury in Aβ<sub>1-42</sub>-treated ARPE-19 cells by repressing the PARP1/SIRT1 pathway.
Disclosure statement
The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Abbreviations
AMD: age-associated macular degeneration
PARP1: poly(ADP-ribose) polymerase
SIRT1: Sirtuin 1
IL-1β: interleukin-1β
IL-6: interleukin-6
IL-8: interleukin-8
TNF-α: tumor Necrosis Factor-α
oAβ: oligomeric amyloid-β
AMD: age-related macular degeneration
ɑ
Abbreviations
AMD: age-associated macular degeneration
PARP1: poly(ADP-ribose) polymerase
SIRT1: Sirtuin 1
IL-1β: interleukin-1β
IL-6: interleukin-6
IL-8: interleukin-8
TNF-α: tumor Necrosis Factor-α
oAβ: oligomeric amyloid-β

ORCID
Yu-jie Tang  http://orcid.org/0000-0002-5999-6610

References
[1] Neely DC, Bray KJ, Huisingh CE, et al. Prevalence of undiagnosed age-related macular degeneration in primary eye care. JAMA Ophthalmology. 2017;135 (6):570–575.
[2] Yuan J. Role of inflammatory factors in the effects of aflibercept or ranibizumab treatment for alleviating wet age-associated macular degeneration. Exp Ther Med. 2019;17(5):4249–4258.
[3] Weng X, Zhang H, Kan M, et al. Leukocyte telomere length is associated with advanced age-related macular degeneration in the Han Chinese population. Exp Gerontol. 2015;69:36–40.
[4] Sharma R, Bose D, Maminishkis A, et al. Retinal pigment epithelium replacement therapy for age-related macular degeneration: are we there yet? Annu Rev Pharmacol Toxicol. 2020;60:553–572.
[5] Simo R, Villarroel M, Corraliza L, et al. The retinal pigment epithelium: something more than a constituent of the blood-retinal barrier—implications for the pathogenesis of diabetic retinopathy. J Biomed Biotechnol. 2010;2010:190724.
[6] Holtkamp GM, Kijistra A, Peek R, et al. Retinal pigment epithelium-immune system interactions: cytokine production and cytokine-induced changes. Prog Retin Eye Res. 2001;20:29–48.
[7] Malais M, De Souto Barreto P, Hooper C, et al. Association between brain beta-amyloid and frailty in older adults. J Gerontol A Biol Sci Med Sci. 2019;74 (11):1747–1752.
[8] Yoshida K, Moein A, Bittner T, et al. Pharmacokinetics and pharmacodynamic effect of crenezumab on plasma and cerebrospinal fluid beta-amyloid in patients with mild-to-moderate Alzheimer’s disease. Alzheimers Res Ther. 2020;12(1):16.
[9] Wang L, Clark ME, Crossman DK, et al. Abundant lipid and protein components of drusen. PLoS ONE. 2010;5:e10329.
[10] Egan MF, Kost J, Tariot PN, et al. Randomized trial of verubecstat for mild-to-moderate Alzheimer’s disease. N Engl J Med. 2018;378(18):1691–1703.
[11] Liu Y, Pan N, Ma Y, et al. Inhaled sevoflurane may promote progression of amnestic mild cognitive impairment: a prospective, randomized parallel-group study. Am J Med Sci. 2013;345(5):355–360.
[12] Chen H, Liu S, Ji L, et al. Folic acid supplementation mitigates Alzheimer’s disease by reducing inflammation: a randomized controlled trial. Mediators Inflamm. 2016;2016:5912146.
[13] Maloney SC, Fernandes BF, Castiglione E, et al. Expression of cyclooxygenase-2 in choroidal neovascular membranes from age-related macular degeneration patients. Retina. 2009;29:176–180.
[14] Cao S, Ko A, Partanen M, et al. Relationship between systemic cytokines and complement factor H Y402H polymorphism in patients with dry age-related macular degeneration. Am J Ophthalmol. 2013;156:1176–1183.
[15] Kauppinen A, Suuronen T, Ojala J, et al. Antagonistic crosstalk between NF-kappaB and SIRT1 in the regulation of inflammation and metabolic disorders. Cell Signal. 2013;25(10):1939–1948.
[16] Zhang YJ, Wu Q. Sulforaphane protects intestinal epithelial cells against lipopolysaccharide-induced injury by activating the AMPK/SIRT1/PGC-1α pathway. Bioengineered. 2021;12(1):4349–4360.
[17] Sim H, Lee W, Choo S, et al. Sulforaphane alleviates particulate matter-induced oxidative stress in human retinal pigment epithelial cells. Front Med (Lausanne). 2021;8:685032.
[18] Yang PM, Cheng KC, Huang JY, et al. 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.
[19] Wang H, Muižnieks LD, Ghosh P, et al. Somatostatin binds to the human amyloid β peptide and favors the formation of distinct oligomers. Elife. 2017;6:e28401.
[20] Wu L, Tan X, Liang L, et al. The role of mitochondria-associated reactive oxygen species in the amyloid beta induced production of angiogenic factors by ARPE-19 cells. Curr Mol Med. 2017;17 (2):140–148.
[21] Masuda N, Tsujinaka H, Hirai H, et al. Effects of concentration of amyloid β (Aβ) on viability of cultured retinal pigment epithelial cells. BMC Ophthalmology. 2019;19(1):70.
[22] Feng L, Liao X, Zhang Y, et al. Protective effects on age-related macular degeneration by activated autophagy induced by amyloid-beta in retinal pigment epithelial cells. Discov Med. 2019;27(148):153–160.
[23] Alemasova EE, Lavrik OI. Poly(ADP-ribosyl)ation by PARP1: reaction mechanism and regulatory proteins. Nucleic Acids Res. 2019;47(8):3811–3827.

[24] Wang S, Han L, Han J, et al. Uncoupling of PARP1 trapping and inhibition using selective PARP1 degradation. Nat Chem Biol. 2019;15(12):1223–1231.

[25] Gibson BA, Kraus WL. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. Nature Reviews Molecular Cell Biology. 2012;13 (7):411–424.

[26] Galluzzi L, Vitale I, Aaronson SA, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 2018;25:486–541.

[27] Chang HC, Guarante L. SIRT1 and other sirtuins in metabolism. Trends Endocrinol Metab. 2014;25 (3):138–145.

[28] O’Callaghan C, Vassilopoulos A. Sirtuins at the cross-roads of stemness, aging, and cancer. Aging Cell. 2017;16(6):1208–1218.

[29] Kauppinen A, Suuronen T, Ojala J, et al. Antagonistic crosstalk between NF-κB and SIRT1 in the regulation of inflammation and metabolic disorders. Cell Signal. 2013;25(10):1939–1948.

[30] Singh V, Ubaid S. Role of silent information regulator I (SIRT1) in regulating oxidative stress and inflammation. Inflammation. 2020;43(5):1589–1598.

[31] Zhou Y, Cai L-J, Xu L-H, et al. The role of sirt1 in the retinal ganglion cells cultured by high glucose. Int Ophthalmol. 2021;41(3):845–852.

[32] Mendelsohn AR, Larrick JW. The NAD+/PARP1/SIRT1 axis in aging. Rejuvenation Res. 2017;20(3):244–247.

[33] Jang KH, Hwang Y, Kim E. PARP1 impedes SIRT1-mediated autophagy during degeneration of the retinal pigment epithelium under oxidative stress. Mol Cells. 2020;43(7):632–644.

[34] Rajamohan SB, Pillai VB, Gupta M, et al. SIRT1 promotes cell survival under stress by deacetylation-dependent deactivation of poly (ADP-ribose) polymerase 1. Molecular and Cellular Biology. 2009;29(15):4116–4129.

[35] Al Sabaani N. Kaempferol Protects Against Hydrogen Peroxide-Induced Retinal Pigment Epithelium Cell Inflammation and Apoptosis by Activation of SIRT1 and Inhibition of PARP1. J Ocul Pharmacol Ther. 2020;36(7):563–577.

[36] Piberger Al, Keil C, Platz S, et al. Sulforaphane inhibits damage-induced poly (ADP-ribose)ylation via direct interaction of its cellular metabolites with PARP-1. Mol Nutr Food Res. 2015;59(11):2231–2242.

[37] Li Y-P, Wang S-L, Liu B, et al. Sulforaphane prevents rat cardiomyocytes from hypoxia/reoxygenation injury in vitro via activating SIRT1 and subsequently inhibiting ER stress. Acta Pharmacol Sin. 2016;37 (3):138–145.

[38] Ye L, Yu T, Li Y, et al. Sulforaphane enhances the ability of human retinal pigment epithelial cell against oxidative stress, and its effect on gene expression profile evaluated by microarray analysis. Oxid Med Cell Longev. 2013;2013:413024.

[39] Sim H, Lee W, Choo S, et al. Sulforaphane alleviates particulate matter-induced oxidative stress in human retinal pigment epithelial cells. Front Med (Lausanne). 2021;8:685032.

[40] Cano V, Reyes M, Park CY, et al. Demonstration by redox fluorometry that sulforaphane protects retinal pigment epithelial cells against oxidative stress. Invest Ophthalmol Vis Sci. 2008;49(6):2606–2612.