• Review Article •

Targeting Nrf2 signaling in dry eye

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Received: 2023-08-22 Accepted: 2024-06-06

Abstract

● Dry eye, the most common ocular surface disease, can cause ocular surface tissue damage and discomfort symptoms and seriously affect people's quality of life. The etiology of dry eye is diverse, and its pathogenesis is complex. The oxidative stress reaction is considered to be among the important factors in the pathogenesis of dry eye. Therefore, activating the antioxidant system has a potential therapeutic effect on dry eye. Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway is considered the most important antioxidant pathway in the body. The activation of the Nrf2 signaling pathway and its interaction with other pathways are important mechanisms to prevent the occurrence and development of dry eye. This review describes the structure and function of Nrf2, summarizes the changes in the oxidative stress response in dry eye, focuses on the potential mechanism of the Nrf2 signaling pathway in the treatment of dry eye, and, finally, summarizes the drugs that activate the Nrf2 signaling pathway in the treatment of dry eye.

● **KEYWORDS:** nuclear factor erythroid 2-related factor 2; Keap1; dry eye; oxidative stress

DOI:10.18240/ijo.2024.10.19

Citation: Zhang YN, Ouyang WJ, Hu JY, Liu ZG. Targeting Nrf2 signaling in dry eye. *Int J Ophthalmol* 2024;17(10):1911-1920

INTRODUCTION

Dry eye is a chronic ocular surface disease caused by multiple factors; this condition can cause symptoms such as eye pain and visual disturbances, damage to the ocular surface, and affect people's quality of life[1]. Tear film instability and increased osmotic pressure, the core mechanisms of dry eye, can induce inflammation and oxidative stress on the ocular surface. A growing body of evidence suggests that oxidative stress may play an important role in dry eye. Clinical and experimental studies have shown that oxidative stress products are increased in the tears and epithelial cells of dry eye patients[2]. Increased oxidative stress products can cause an inflammatory response[3]. Therefore, inhibiting oxidative stress has become a new direction for the treatment of dry eye.

Regulating the expression of many antioxidants in cells is the main fuction of nuclear factor erythroid 2-related factor 2 (Nrf2). To date, it has been confirmed that Nrf2 can upregulate nearly 20 antioxidant genes and regulate more than 200 protective genes overall. Therefore, Nrf2 has biological effects such as maintaining the cellular oxidation-antioxidative balance, inhibiting apoptosis, regulating immunity, and inhibiting inflammation, and it plays an important role in antitumor, antiaging, and antiatherosclerotic processes[4]. Recent studies have found that the Nrf2 signaling pathway, as a key factor regulating oxidative stress, showed a range of potential benefits in treating dry eye. Therefore, this paper describes the structure and function of Nrf2, summarizes the changes in oxidative stress in dry eye, focuses on the potential mechanism of the Nrf2 signaling pathway in treating dry eye, and, finally, summarizes the drugs that may treat dry eye by activating the Nrf2 signaling pathway, providing new ideas for the treatment of dry eye.

Overview of the Nrf2 Signaling Pathway

Structure and regulation of Nrf2 Nrf2 is a Cap'n'collar (CNC) basic leucine zipper (bZIP) transcription factor composed of seven functional domains, Nrf2-ECH homology 1 (Neh1) to Neh7[5]. The Neh1 domain is located within the C-terminus of Nrf2, and its primary function is to bind

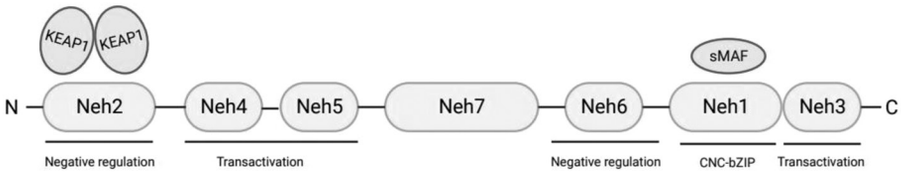

Figure 1 Protein domains of the transcription factor Nrf2 Nrf2 is composed of seven Neh domains. Neh1 is the CNC-bZIP domain that interacts with sMaf proteins. The Neh2 and Neh6 responsible for the negative regulation of Nrf2. Neh3, Neh4, and Neh5 are important for transactivation. The Neh7 domain interact with retinoid X receptor alpha to inhibit the transcriptional activity of Nrf2. Neh: Nrf2-ECH homology; CNC: Cap'n'collar; bZIP: Basic leucine zipper; sMaf: Small maf. Created by Biorender.

the sMaf (small maf) protein, DNA and other transcription-related molecules[6]. The Neh2 domain, which is located in the N-terminal region, binds to Kelch-like ECH-associated protein 1 (Keap1), can mediate the degradation of Nrf2 by the proteasome. Therefore, it is the most significant regulatory domain[7]. The Neh3 domain is located within the C-terminus; it has transactivation activity and can interact with the Neh4 and Neh5 domains to transactivate Nrf2 target genes[8]. Neh4 and Neh5, two activation domains between Neh1 and Neh2, have been demonstrated to promote the transactivation of Nrf2 target genes[9]. The Neh6 domain is a serine-rich region that is involved in the Keap1-independent negative regulation of Nrf2 stability. It regulates Nrf2 stability[10]. The Neh7 domain is located within the center of Nrf2 and was shown to interact with retinoid X receptor alpha to inhibit the transcriptional activity of Nrf2[11] (Figure 1).

Under basal conditions, Nrf2 physically interacts with the negative regulator Keap1[12] and inhibits Nrf2 activation by binding to a substrate of the cullin 3 E3 ubiquitin ligase complex (Cul3) to promote Nrf2 ubiquitination and subsequent proteasomal degradation[13]. During oxidative stress or exposure to Nrf2 activators, Nrf2 dissociates from Keap1 conjugates due to thiol modification of Keap1 cysteine residues, ultimately preventing Nrf2 ubiquitination and proteasomal degradation. Nrf2 then translocates to the nucleus, and the Neh1 domain binds sMaf proteins to form heterodimers, activating the genes regulated by the antioxidant response element (ARE)[14].

Nrf2 and Oxidative Stress

Under physiological conditions, oxidative phosphorylation produces a few reactive oxygen species (ROS) and reactive nitrogen species (RNS)[15]. They are significant molecules with physiological functions. They regulate cell division, inflammation, immunity, *etc*., and they do not disturb the connection between Keap1 and Nrf2. Under stress, excess ROS will be generated[16-17], vitiating cellular function. In response to stress, Keap1 is inactivated, which causes the destabilization of Nrf2 and the arrest of Nrf2 degradation, resulting in rapid accumulation of Nrf2. Keap1 inactivates and releases Nrf2. Nrf2 is then transferred into the nucleus and forms a heterodimer with the sMaf protein to induce the transcription of Nrf2-responsive antioxidant genes [heme oxygenase-1 (HO-1), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), *etc.*][18-19]. Evidence suggests that Nrf2 knockout mice are more sensitive to oxidative stress than wild-type mice, and the expression of antioxidant genes is reduced through ARE regulation after Nrf2 knockout[20].

Nrf2 also modulates antioxidant defense in response to oxidative stress through multiple mechanisms and directly acts upon ROS and RNS homeostasis. It can induce superoxide and peroxide catabolism[21], regenerate oxidative proteins[22], stimulate the synthesis reducing factors[23], drive expression of the antioxidant protein thioredoxins (Trx), inhibit the expression of the Trx inhibitor thioredoxins-interacting protein (TXNIP)[24], induce the cystine/glutamate antiporter SLC7A11 (also commonly known as xCT) expression[25], promote the separation of metals from melatonin receptors and the chelation of iron from ferritin[26], and induce the expression of antioxidant proteins[27].

Many stress-responsive proteins regulated by Nrf2 localize to particular regions inside the cell, allowing redox signaling to be regulated in the local environment, and they also regulate the expression of several oxidation signaling proteins that affect several programmed cellular functions[28]. Some regulatory factors, such as protein sequestosome 1/p62 (p62)[29] and protein deglycase DJ-1 (Parkinson disease protein 7)[30], activate and induce Nrf2 through oxidants, forming a positive feedback loop with Nrf2[27].

Overall, activation of Nrf2 is effective in responding to oxidative stress[31].

Dry Eye and Nrf2

Increased oxidative products in dry eye The human eye, especially the surface of the eye, is easily damaged by oxidative stress because it is an organ in direct contact with the external environment. Factors such as age, environment (particles, gases, ozone), and benzalkonium chloride increase ROS levels in the corneal epithelium[2]. The increase in ROS reduces the activity of cellular breakdown and repair mechanisms, leading to the accumulation of oxidized molecules and the formation of amyloid, which disrupts the function of corneal epithelial cells.

Recent studies have shown that oxidative stress damage plays an important role in the pathogenesis of dry eye[3]. Exposure to a dry environment will disrupt the balance of the ocular surface oxidation-antioxidation system, reducing the levels of key reductases (SOD, GSH-Px, CAT) and oxidative stress products that resist oxidative stress. ROS levels may increase ROS damage to macromolecules such as proteins, lipids, and DNA, resulting in damage to ocular surface tissues[32]. Disruption of ocular surface epithelial barrier function and reduced blinking have also been shown to be associated with oxidative stress in experimental dry eye. In addition, with the gradual increase in irritation that occurs as a symptom of dry eye, the conjunctival epithelial nitric oxide synthase levels of dry eye patients also gradually increased, and the same was true of inflammatory factors [interleukin (IL-1β), IL-6, IL-8, tumor necrosis factor-α (TNF-α), *etc.*][33]. These findings suggest that oxidative stress is involved in the pathogenesis of dry eye.

While the lacrimal gland is not repeatedly attacked by environmental changes in the same way as the surface of the eye, it undergoes age-related changes. Many external factors as well as immune system dysregulation can lead to a lifelong inappropriate stress response that can lead to inflammation and structural changes in the tear ducts. Oxidative stress in the lacrimal gland leads to tear film dysfunction and the development of dry eye[34].

This study compared oxidative stress levels in individuals with and without dry eye. Compared with healthy controls, the levels of lipid peroxidation products, myeloperoxidase, nitric oxide synthase, xanthine oxidase/oxidoreductase, 4-hydroxynonenal (4-HNE), malondialdehyde, reactive oxygen species, and oxidative stress markers were generally elevated in patients with dry eye. Oxidative stress markers in tears, conjunctival cells, and conjunctival biopsies were higher in dry eye patients than in control[35].

Regulation of oxidative stress in dry eye by Nrf2 The cornea and conjunctiva are the structures on the ocular surface that come directly into contact with external factors[36]. The high expression of Nrf2 in epithelial cells allows the cornea and conjunctiva to have a positive defense against oxidative stress[37].

In a model of smoke exposure, Nrf2 knockout mice showed a shorter tear film break-up time (TBUT) and more severe corneal fluorescein sodium staining than wild-type mice. Later studies showed that 8-hydroxydeoxyguanosine (8-OHdG) and 4-HNE were significantly elevated in the cornea and conjunctiva of Nrf2 knockout mice[38]. In addition, the use of esculetin (an Nrf2 agonist) significantly induced Nrf2 expression and upregulated downstream antioxidant genes such as HO-1, NQO1 [NAD(P)H: quinone oxidoreductase 1], SOD1, and SOD2 and improved dry eye symptoms in a mouse model of dry eye[39].

These results suggest that Nrf2 plays an important role in maintaining the level of oxidative stress in ocular surface tissue during dry eye and may be a potential target for the treatment of dry eye.

Mechanism by Which Nrf2 Regulates the Pathogenesis of Dry Eye

Inhibition of chronic inflammatory response by Nrf2 Nrf2 is key to fighting oxidative stress and is known to reduce inflammation. Nrf2 deficiency leads to increased inflammation in mouse models of various conditions, such as sepsis, pleurisy, and emphysema. Activation of Nrf2 in myeloid cells consistently reduces inflammation. The Nrf2 inducer Tecfidera (dimethyl fumarate) has been partially approved for the treatment of multiple sclerosis due to its anti-inflammatory properties in human clinical studies. These observations suggest that Nrf2 is critical for the control of inflammation[18].

Nrf2 directly controls the expression of the HMOX1 gene encoding the HO-1 enzyme. Activation of the Nrf2/HO-1 axis reduces lipid peroxidation as well as TNF-α and IL-6 levels[40]. Several *in vitro* and *in vivo* experiments have demonstrated the critical role of Nrf2-mediated HO-1 expression in anti-inflammatory activity[41].

Nrf2 can also achieve anti-inflammatory effects by modulating the NF-kB signaling pathway[42]. NF-kB is also a transcription factor that regulates redox action and has regulatory effects on both the inflammatory response and cell injury. After nuclear translocation, NF-kB induces the expression of inflammatory cytokines (IL-1, IL-6, TNF-α), cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), vascular adhesion molecules, *etc*[43]. Functionally, Nrf2 downregulates NF-κB signaling through multiple mechanisms. First, Nrf2 inhibits oxidative stress-mediated activation of NF-κB and reduces intracellular ROS levels[44]. Furthermore, Nrf2 inhibits the proteasomal degradation of IκB-α and inhibits the nuclear translocation of NF-κB. Upregulation of Nrf2 induces increased cellular levels of HO-1, which, in turn, increases the expression of phase II enzymes that prevent IκB-α degradation[45].

Nrf2 also regulates the NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome in the following ways. Since Nrf2 is involved in the regulation of oxidative stress and antioxidant gene expression, it is thought to inhibit the activation of NLRP3 by inhibiting the generation of ROS[46]. In addition, Nrf2 inhibits the activity of the NLRP3 inflammasome by reducing the expression of genes related to inflammasome assembly, such as NLRP3, Caspase1, IL-1β, and IL-18, and thus inhibits inflammation[47]. Calcitriol, an activator of Nrf2, inhibits the ROS-NLRP3-IL-1β signaling axis, thereby reducing the expression of NLRP3

inflammasome-related genes and the production of IL-1β in cells exposed to hyperosmolarity. In this way, calcitriol protects cells from cellular inflammation induced by a hypertonic environment[48].

Inhibition of chronic pain by Nrf2 Dry eye-associated sensory nerve abnormalities and neuropathic pain are highlighted in TFO DEWS II[1]. In dry eye disease, decreased tear production can lead to inflammation and peripheral nerve damage. Inflammation causes not only multimodal and mechanoreceptor sensitization of nerve endings but also abnormally increased activity of heat and cold thermoreceptors, which collectively lead to dryness and pain. Cell bodies in the trigeminal ganglion and brainstem, altering their excitability, connectivity and impulsivity. The persistent impairment of ocular sensory pathways at the molecular, structural and functional levels leads to ocular surface neuropathy and neuralgia[49]. Therefore, new requirements are put forward for the treatment of dry eye.

Various Nrf2 activators (*e.g.*, sulforaphane, oltipraz) have been studied for their analgesic effects[50-51]. Several studies have shown that Nrf2 activation can reduce acute and chronic inflammatory pain[50] and diabetes-associated neuropathic pain[52] induced by nerve injury or chemotherapy. Nrf2 activators promote increased or normalized expression of Nrf2, HO-1, NQO1, glutathione transferase, or superoxide dismutase and inhibit microglial activation in acute and chronic pain[53-54]. In animals with type 2 diabetes, sulforaphane stimulated Nrf2 and HO-1 expression in the sciatic nerve and restored the hyperglycemia-induced downregulation of NQO1. The expression of Nrf2 and HO-1 was decreased in the spinal cord, amygdala, prefrontal cortex, and hippocampus of animals with neuropathic pain induced by sciatic nerve injury[55], and the activation of Nrf2 and the promotion of HO-1 expression alleviated neuropathic pain induced by sciatic nerve damage. Conversely, the loss or disruption of Nrf2 signaling increases susceptibility to oxidative stress and inflammatory damage[56]. These studies suggest that Nrf2 activators can suppress oxidative stress and chronic inflammation by activating the Nrf2/HO-1/NQO1 signaling pathway, thereby alleviating neuropathic pain and diabetic neuropathy[50,57].

Promotion of Nrf2 on corneal epithelial injury repair The pathophysiological process of dry eye is related to corneal epithelial cell apoptosis and epithelial cell shedding, and the clinical manifestation is positive staining of corneal fluorescein sodium. Therefore, promoting the proliferation and migration of corneal epithelial cells contributes to the healing of corneal epithelial lesions in dry eye disease. The Nrf2-mediated defense system plays a key role in protecting cells by activating genes to protect against such stress. Studies have shown that the Nrf2-mediated defense system plays an important role in the healing of corneal epithelial wounds. Using a mouse corneal epithelial scraping model, it was found that corneal epithelium from Nrf2-KO mice showed significantly delayed healing, whereas corneal epithelium from wild type (WT) mice showed Nrf2 activation. Ki-67 staining revealed that the number of Ki-67-positive proliferating cells was significantly lower in Nrf2-KO mice than in WT mice 24-36h after injury. Knockdown of Nrf2 *in vitro* delayed the migration of corneal epithelial cells but did not affect cell proliferation. Conversely, a reduction in the expression of the Keap1 gene can be shown to increase the speed of cell migration. These findings suggest that Nrf2 participates in the corneal epithelial wound healing process by accelerating cell migration[58]. Therefore, the activation of Nrf2 may achieve the purpose of treating dry eye by promoting the proliferation of corneal epithelial cells.

In summary, Nrf2 can achieve the purpose of treating dry eye by inhibiting chronic inflammation, pain and promoting the repair of corneal epithelial injury (Figure 2).

Therapeutic Potential of Nrf2 Inducers in Dry Eye

Esculetin Esculetin (6,7-dihydroxycoumarin), a natural product that exists in a variety of plants, fungi and bacteria, has been reported to have a variety of pharmacological actions, including antioxidant, antibacterial and other pharmacological effects. Esculetin has been widely used as a complementary and alternative medicine; it is considered a promising drug[59]. In dry eye, for example, esculetin has strong therapeutic potential. One study reported that treatment with esculetin and cyclosporine A alleviated dry eye symptoms by regulating the expression levels of inflammatory cytokines (*e.g.*, IL-1α, IL-1β, TNF-α) in a rabbit model of dry eye after lacrimal duct excision[60]. Studies have shown that esculetin effectively alleviates H_2O_2-induced oxidative damage in human corneal epitheliumcells (HCECs) through Nrf2 signaling pathway–related antioxidant properties. In addition, in a mouse model of dry eye, esculetin significantly ameliorated dry eye symptoms[39].

Calcitriol Calcitriol [calcitriol,1α,25-$(OH)^2$D3] is the active metabolite of vitamin D; its main function is to regulate calcium and phosphate homeostasis, and it also regulates cell proliferation and differentiation and plays a key role in the reactions of the immune and nervous systems[61]. The mechanism of action of calcitriol is mediated by vitamin D receptors, a subfamily of nuclear receptors that enter target cells as transcription factors after heterodimerization with retinoid X receptors. These receptors are found in nearly all cell types, which may explain their diverse effects on different tissues[62]. The currently known effects of calcitriol include xenobiotic detoxification, reduction of oxidative stress, neuroprotective function, antibacterial defense, immunomodulation, anti-inflammatory/anticancer effects

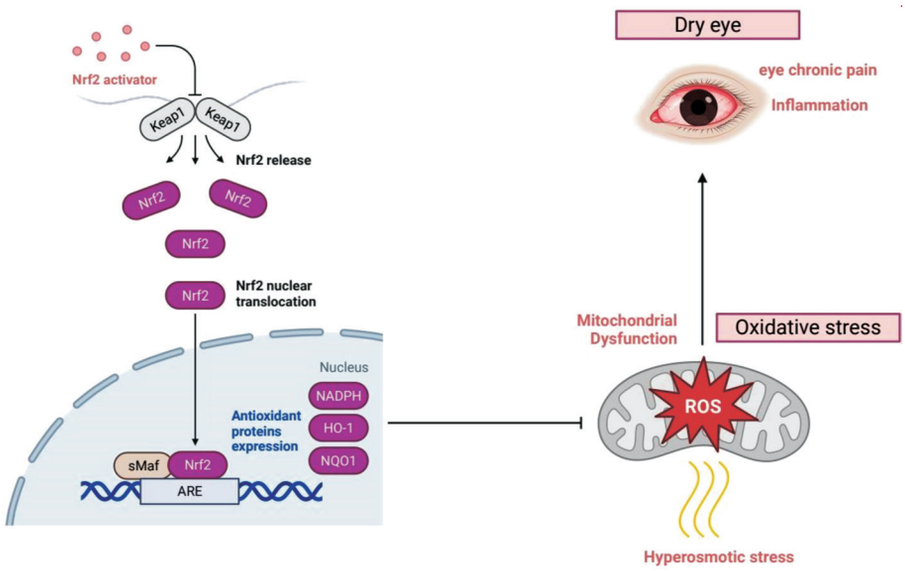

Figure 2 The role of the Nrf2 signaling pathway Various compounds can act as Nrf2 activators, *i.e.*, induce the activation of the Nrf2 signaling pathway. Keap1 is inactivated, resulting in the destabilization of Nrf2 and the arrest of Nrf2 degradation, resulting in rapid accumulation of Nrf2. Nrf2 subsequently translocates into the nucleus, forms a heterodimer with the sMaf protein, and binds AREs to activate antioxidant genes (HO-1, NADPH, NQO1, *etc.*). These antioxidant genes can effectively alleviate mitochondrial dysfunction caused by hyperosmotic stress, thereby inhibiting chronic inflammation and chronic pain in dry eye and promoting the repair of corneal epithelial injury. sMaf: Small maf; AREs: Antioxidant response elements; HO-1: Heme oxygenase-1; NADPH: Nicotinamide adenine dinucleotide phosphate; NQO1: NAD(P)H:quinone oxidoreductase 1. Created by Biorender.

and cardiovascular benefits, and it has been used as a basis to develop various drugs due to its wide range of physiological activity[63]. It is mainly used for the treatment of osteoporosis, kidney disease, skin disease and immune-related diseases in the clinic[64].

Studies have shown that calcitriol can protect cells from *hyperosmolality*-induced damage by inducing the translocation of Nrf2 to the nucleus, triggering the expression of various antioxidant enzymes, and inhibiting the ROS-NLRP3-IL-1β signaling axis. Calcitriol significantly inhibited the expression of NLRP3 inflammasome-related genes and the production of IL-1β in hyperosmotic stress (HS)-exposed cells. Calcitriol also significantly attenuated HS-induced oxidative stress, manifested as decreased intracellular ROS generation and decreased 8-OHdG-stained cells[65]. Other studies have confirmed that calcitriol can significantly improve dry eye symptoms and reduce corneal inflammation at the animal level, and at the cellular level, calcitriol also significantly reduces hyperosmotic stress by inhibiting NF-κB activation. Expression of proinflammatory mediators in HCECs gets reduced[66].

Therefore, calcitriol might inhibit the initiation phase of HS-induced cellular inflammation, highlighting its potential ability to prevent and alleviate dry eye-related corneal inflammation early. Calcitriol has the ability to control dry eye-related inflammation at the initiating step; thus, it may be a promising therapeutic agent for dry eye[48].

Oltipraz Oltipraz, a sulfhydryl compound extracted from cruciferous vegetables, is an organosulfur compound belonging to the dithiolthione group. Several studies have confirmed that oltipraz can induce Nrf2 activation and has antioxidant properties[67-70]. There are studies showing its therapeutic potential in dry eye. Adding oltipraz to the diet of aged mice significantly improved the pathological changes in the lacrimal gland, decreased nitrotyrosine and 4-HNE, decreased the expression of IL-1β and TNF-α, and significantly increased the density of conjunctival goblet cells. These findings provide new insights into the development of chronic, low-grade inflammation and oxidative stress in age-related dry eye, and new therapies targeting oxidative stress pathways are valuable for the treatment of age-related dry eye[34].

Novel triterpenoid RS9 (a biotransformation product of RTA 402) RS9 [C32H43NO6; methyl(1a,2a,21b)-2-cyano-21-hydroxy-3,12-dioxo-1,2-epoxyo-lean-9(11)-en-28-oate] is a novel derivative of the raw material bardoxolone methyl (BARD), or methyl(1a,2a,21b)-2-cyano-21-hydroxy-3,12-dioxo-1,2-epoxy-olean-9(11)-en-28-oate. Compared with BARD, RS9 has increased NQO1-inducing activity and reduced cytotoxicity[71].

Studies have used *in vitro* and *in vivo* models to evaluate the efficacy of RS9 on dry eye. The results showed that topical application of RS9 significantly improved changes in various oxidative indicators. RS9 induces Nrf2 activation and Nrf2-targeted genes, reduces oxidation, and improves symptoms in

Table 1 The therapeutic potential of Nrf2 inducers in dry eye

Nrf2 inducer	Models	Changes in oxidative indicators and dry eye indicators	References
Esculetin	Hyperosmotic stress, dry stress induced dry eye model	ROS↓, Nrf2↑, HO-1↑, GPx1↑, GCLC↑; corneal injury↓, epithelial cell apoptosis↓; goblet cell number↑	39
Calcitriol	Hyperosmotic stress, BAC-induced dry eye model	NLRP3↓, TBUT↑, tear production↑, the fluorescein staining score↓, IL-6↓, IL-1β↓, TNF-α↓, NF-kB↓	65-66
Oltipraz	Nrf2 knockout mouse	Nitrotyrosine↓, 4-HNE ↓	34
The novel triterpenoid RS9	Hyperosmotic stress, Scopolamine-induced dry eye model	ROS↓, Nrf2↑, HO-1↑, GPx1↑, GCLC↑, 8-OHdG↓	72
Sulforaphane (SFN)	None	None	50
Trehalose	None	None	86
Edaravone	Hyperosmotic stress	ROS↓, Nrf2↑ HO-1↑, GPx-1↑, GCLC↑	78
Dimethyl fumarate	None	None	85

ROS: Reactive oxygen species; Nrf2: Nuclear factor erythroid 2-related factor 2; HO-1: Heme oxygenase-1; Gpx1: Glutathione peroxidase 1; GCLC: Glutamate-cysteine ligase; NLRP3: NOD-like receptor thermal protein domain associated protein 3; TBUT: Tear film break-up time; 4-HNE: 4-Hydroxynonenal; 8-OHdG: 8-Hydroxydeoxyguanosine. ↑: Increase; ↓: Decrease.

in vitro and in vivo models of dry eye. Therefore, RS9 may be an effective drug candidate against dry eye disease[72].

Sulforaphane Sulforaphane is an isothiocyanate found in a stored form in cruciferous vegetables such as cabbage, cauliflower, and kale. Sulforaphane is metabolized *via* the thiol acid pathway; it is coupled to glutathione and undergoes further biotransformation, producing metabolites. Sulforaphane has been extensively studied and has been found to protect against various types of cancer[73], reduce the risk of cardiovascular disease, and contribute to autism and osteoporosis[74]. Sulforaphane, an activator of Nrf2[75], can alleviate chronic inflammatory pain by promoting the expression of Nrf2, HO-1, NQO1, inhibiting inflammation-induced overexpression of iNOS and CD11b/c and mitogen-activated protein kinase (MAPK) phosphorylation and enhancing the analgesic effects of morphine[50]. Based on this, sulforaphane may also treat dry eye by activating Nrf2, thereby inhibiting inflammatory responses induced by oxidative stress and peripheral inflammation.

Edaravone Edaravone is a hydroxyl radical scavenger[76] that is used clinically to reduce neuronal damage after ischemic stroke[77]. Edaravone also increased the expression of Nrf2 and its target genes HO-1, glutathione peroxidase 1 (GPx-1) and glutamate-cysteine ligase (GCLC).We found that edaravone reduced ROS levels and mitochondrial oxidative damage in a dose-dependent manner in a hyperosmotic mediator-induced cellular model. Furthermore, edaravone can improve mitochondrial function by increasing adenosine triphosphate (ATP) levels and mitochondrial membrane potential, reducing caspase-3 levels and inhibiting cytochrome C release to inhibit apoptosis[78]. This demonstrates the protective effect of edaravone in a hypertonic culture model of HCECs. These findings strongly suggest that edaravone may be a potential drug candidate for the treatment of dry eye.

Trehalose Trehalose, the disaccharide of glucose, is a nontoxic and nonreducing bioactive sugar naturally found in marine organisms. Trehalose has been shown to promote wound healing by protecting cell membranes from oxidative damage and desiccation[79]. Trehalose inhibits corneal inflammation, scarring and corneal neovascularization. In dry eye, trehalose can activate Nrf2 expression, reduce apoptosis and reduce oxidative, inflammatory and proteolytic activity of the ocular surface, improving symptoms of dry eye[80]. Clinical trials have demonstrated that trehalose is more effective than 0.1% Hyaluronic Acid eye drops in the treatment of dry eye[81].

Dimethyl fumarate Dimethyl fumarate (DMF) is a small molecule approved by the US Food and Drug Administration (FDA) for the treatment of multiple sclerosis (MS)[82]. The mechanism of action of DMF is not fully understood but has been shown to activate Nrf2 in some studies, such as in rheumatoid arthritis[83]. DMF can improve rheumatoid arthritis by activating the Nrf2/HO-1 pathway and inhibiting the generation of ROS and the expression of IL-6, IL-33, and matrix metalloproteinase (MMP) 1/3/9[84]. DMF also reduces the severity of optic neuritis and preserves vision and retinal ganglion cell (RGCs)[85]. Based on this, DMF can effectively activate Nrf2, inhibit oxidative stress and the inflammatory response, and can be used as a potential therapeutic drug for dry eye (Table 1)[34,39,50,65-66,72,78,85-86].

CONCLUSION AND OUTLOOK

Oxidative stress is an important mechanism in the pathogenesis of dry eye, and studies have shown that controlling oxidative stress can effectively improve dry eye symptoms. As the most important pathway in the body's antioxidant mechanism, the Nrf2 pathway is closely linked to the onset and progression of dry eye disease. The activation of this signaling cascade and its interaction with other pathways are important mechanisms to stop dry eye from happening and progressing. Therapy targeting this mechanism is expected to become a new prospect for the treatment of dry eye. Many scholars have conducted

research on Nrf2 agonists in the treatment of dry eye and achieved remarkable results. However, there are currently no clinical trials to verify their effectiveness, and the interaction between Nrf2/Keap1 signaling and other signaling pathways and the exact mechanism that affects the release of Nrf2 are still unclear. Further research will provide a direction for in-depth study of the relationship and mechanism of Nrf2/Keap1 antioxidant system and the occurrence and development of dry eye, as well as clinical drug research.

ACKNOWLEDGEMENTS

Authors' contributions: Zhang YN and Ouyang WJ wrote the article. Hu JY: Resources; supervision; writing–review and editing. Liu ZG: Resources; supervision; writing–review and editing. All authors contributed to the article and approved the submitted version.

Foundations: Supported by the National Natural Science Foundation of China (No.82271054; No.U20A20363).

Conflicts of Interest: Zhang YN, None; **Ouyang WJ,** None; **Hu JY,** None; **Liu ZG,** None.

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