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Bioactive peptides alleviating oxidative stress-associated diseases by targeting the Nrf2 signaling pathway

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ABSTRACT

Oxidative stress is caused by an imbalance of the antioxidant defense system and excessive production of free radicals, which can damage biological macromolecules such as proteins, lipids and nucleic acids, and can cause aging, ischemia-reperfusion injury, inflammatory injury, liver and kidney injury and other diseases. The nuclear factor erytheroid-2-related factor 2 (Nrf2) is a major regulator of redox balance. The Nrf2 pathway also exerts a central function in cell apoptosis, oxidative stress damage and metabolic diseases. Bioactive peptides are small molecular peptides composed of amino acid residues. They have many biological activities and play important physiological roles in human body. Antioxidant peptide is a kind of peptide which can reduce oxidative stress damage. They are safe, non-toxic and easily absorbed. In this review, we summarized the mechanism of bioactive peptides in regulating oxidative stress, especially antioxidant peptides, through regulating the Nrf2 signaling pathway and the expressions of oxidative stress-related genes, to alleviate oxidative stress-induced damage. The paper will provide valuable reference to investigators in the antioxidant peptide field and also promote the applications of antioxidant peptides in the oxidative stress-associated diseases.

1. Introduction

The over-productions of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells and tissues are the main causes of the imbalance between oxidative and antioxidant systems, which cause oxidative stress damage[1]. Oxidative stress is a hazard to biological molecules including proteins, lipids and nucleic acids, and interrupts the transmission of redox signals and causes damage[2]. Oxidative stress damage is closely related to many diseases, these include ischemia-reperfusion (I/R) injury, hypertension, diabetes, inflammatory damage, liver damage, arteriosclerosis, Alzheimer's disease (AD), aging and cancer. It is very important to understand the mechanisms of oxidative stress damage in the above diseases and benefits the treatment and prevention of oxidative stress-related diseases.

There are some antioxidant enzymes in the body, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), thioredoxin reductase. These enzymes are important components of the antioxidant system, which can effectively eliminate excess free radicals, thereby reducing oxidative stress-caused damages[3]. Nuclear factor erytheroid-2-related factor 2 (Nrf2) is a key transcription factor that regulates the expressions of these antioxidant enzymes. For example, Nrf2 could regulate the expression of heme oxygenase-1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (NQO1), and reduce ROS levels in the oxidative stress response[4].

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Meanwhile, Nrf2 also increased the expressions anti-apoptotic proteins and reduced cell damage[5]. Overall, Nrf2 plays a central role in mitigating oxidative stress damage, which provides a valuable intervention target for the preventions and treatments of patients with oxidative stress diseases.

Bioactive peptides are composed of 20 natural amino acid residues with different composition and arrangement. The molecular weight of bioactive peptides is usually less than 6 000 Da, which has many biological functions such as antibacterial, antihypertensive, antioxidation and anti-inflammation[6]. Many synthetic antioxidants on the market are limited in their applications in food processing due to their toxic side effects[7-10]. Natural antioxidant peptides have been paid more and more attention because they are non-toxic and easy to be digested and absorbed by human body[11]. Natural antioxidant peptides, mainly derived from animals, plants and microorganisms, can reduce oxidative stress-caused damage[12]. For example, bioactive peptides obtained from *Spirulina platensis* exhibited stronger free radical scavenging activities by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation and 1,1-diphenyl-2-picrylhydrazyl (DPPH)assays[13]. Peptides SeP2 and SeP5 isolated from golden cuttlefish could increase SOD activity and decreased the contents of ROS and malondialdehyde (MDA), which reduced oxidative stress-induced damage in the nematodes[14]. Our previous publication indicated that active peptides F2d from rice protein could enhance SOD activity and reduced MDA and ROS contents in the hydrogen peroxide (H_2O_2) -induced human umbilical vein endothelial cells (HUVECs). F2d could decrease oxidative damage by activating the Kelch-like ECH-associated protein 1 (Keap1)/Nrf2 signaling pathway. Meanwhile, F2d also increased the expression of B-cell lymphoma-2 (Bcl2) and inhibited the expression of Bcl2-associated X (Bax), which could reduce oxidative stress-induced apoptosis of HUVECs in mitochondrial-dependent manner[15]. Yak derived peptide alleviates H_2O_2-induced oxidative stress by regulating Nrf2 signaling pathway[16-18]. In summary, active peptides targeting Nrf2 signaling pathway can reduce to oxidative stress-caused damage, which provides new clue and promising interventions for the therapy and prevention of oxidative stress-related diseases. This article introduces the oxidative stress reaction and its mechanism. We focus on active peptides attenuate oxidative stress injury by targeting Nrf2 pathway. In addition, we also explore the research progress of the bioactive peptides that target the Nrf2 pathway and regulate oxidative stress-related diseases.

2. Peptides and oxidative stress

2.1 Oxidative stress

The phrase “oxidative stress” was first used in connection with rubber chemistry in 1956[19]. Now, the term oxidative stress refers to a state in which the excessive production of ROS and RNS leads to the imbalance of oxidation and anti-oxidation in the body, such as peroxynitrite ($ONOO^-$), H_2O_2, hydroxyl radical and superoxide anion radical[20]. Oxidative stress can cause oxidative damage to cells and organs[21]. Oxidative stress can damage nucleic acids, proteins, lipids, carbohydrates and other biological macromolecules, and cause several diseases, including cancer, neurodegenerative illnesses, diabetes and obesity[22-26]. Many specific organelles, including mitochondria, golgi apparatus (GA), endoplasmic reticulum (ER), and others, are involved in the complex biochemical reaction known as oxidative stress. These organelles have the capacity to trigger adaptive responses to oxidative stress, protect cells from oxidative damage and re-establish the balance between oxidants and antioxidants[27-29]. Mitochondria are the main targets of ROS[30]. When cells are damaged by oxidative stress, a large amount of ROS is produced in mitochondria, and the accumulation of ROS can lead to mitochondrial dysfunction, thereby activating Bax expression[31]. Bax enhances mitochondrial permeability by interacting with some proteins on the mitochondrial membrane. Damage to the mitochondrial membrane causes to the release of cytochrome c, which activates a series of Caspase and leads to cell apoptosis. The redox homeostasis of the ER will be disturbed by the overproduction of ROS, further leading to protein misfolding and aggregation[32], thus activating endoplasmic reticulum stress (ERS) and causing cell apoptosis[33]. Oxidative stress can cause disruption of Ca^{2+} homeostasis in GA, and intracellular Ca^{2+} overload will stimulate the expressions of Caspase family, which causes GA fracturing and apoptosis[34]. Oxidative stress promotes ROS production by regulating the expression of golgi phosphoprotein 3 in the GA, leading to cell apoptosis.

2.2 Singling pathways regulated by peptides to respond oxidative stress

It has been reported that bioactive peptides attenuate oxidative stress-induced injury by regulating various signaling pathways, such as Keap1/Nrf2/ARE, nuclear factor-κB (NF-κB), phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) and mitogen activated protein kinase (MAPK) signaling pathways.

2.2.1 Keap1/Nrf2/ARE signaling pathway

Keap1/Nrf2/ARE signaling pathway can activate antioxidant responses and significantly reduce oxidative stress-induced damage. Many publications indicate that various peptides have antioxidant functions by triggering Nrf2 signaling pathway. For example, peptide-P60 derived from rice could alleviate oxidative damage through modulating Nrf2 activity[35]. Yi et al.[36] found that peptides isolated from soy could reduce oxidative stress in H_2O_2-induced HepG2 cells by activating Nrf2 pathway and reducing ROS and MDA contents through increasing SOD and catalase (CAT) activities. How do bioactive peptides regulate the Keap1-Nrf2 complex? Some peptides have been reported to displace Nrf2 and can competitively bind to Keap1, thereby inhibiting the formation of Keap1-Nrf2 complexes and allowing Nrf2 to enter the nucleus, which can promote the gene expressions of exert antioxidant effects. Interestingly, molecular docking analysis also approve the result. For example, anti-oxidant peptides GWY and QWY could compete with Nrf2 for binding to the residues Arg415, Arg483, Arg380 and Ser555 in the active sites of Keap1[37]. In another study, Wang et al.[38] obtained peptide Glu-Asp-Tyr-Gly-Ala (EDYGA) from soft-shelled turtle. Molecular docking results showed that Arg415 and Arg380 of Keap1 Kelch domain could bind to EDYGA amino acid residues “Glu” and “Gly” to form hydrogen bonds. In addition, disrupting Keap1-Nrf2 interaction was observed with peptides extracted from cottonseed proteins[39]. Similarly, peptides NTVPAKSCQAQPTTM (N-15-M), QGPIVLNPWDQVKR (Q-14-R) and APSFSDIPNPIGSENSE (A-17-E) were extracted from

fermented milk protein could bind Keap1 and dissociate Nrf2, and Nrf2 could enter nucleus to promote the gene expressions of antioxidant effect[40]. In this study, Ser3, Asn9 and Glu14 of A-17-E were bond to Ser363, Arg415, Tyr572 and Arg336 of Keap1 Kelch domain. Asn7, Asp10 and Gln11 of peptide Q-14-R were bond to Asn387, Arg380, His432, Tyr525, Arg415 and Ser508 of Keap1 Kelch domain. Thr2, Lys6, Gln11 of peptide N-15-M were bond to Ser602, Gly433, Ile435 and Thr576 of Keap1 Kelch domain. These studies suggest that bioactive peptides can regulate Keap1-Nrf2 signaling pathway and participate anti-oxidative stress.

2.2.2 NF-κB signaling pathway

NF-κB is also called transcript factor of oxidative stress and oxidative stress can activate NF-κB. Many peptides can inhibit NF-κB signaling pathway and reduce oxidative stress-induced damage. Rice derived peptide AAGALPS could prevent NF-κB nuclear translocation and inhibited NF-κB activation, which reduced the expression of tumor necrosis factor-α (TNF-α) in the oxidative stress-induced HUVECs[41]. In another study, researchers obtained two new peptides SPP-1 (QLGNLGV) and SPP-2 (SVMPVVA) from seaweed pointed billed fish, and found that both peptides could reduce H_2O_2-induced oxidative stress damage in human dermal fibroblasts (HDF)[42]. In addition, comparing with H_2O_2 treatment, the peptide treatment reduced ROS content and increased the levels of SOD1, GSH and CAT in HDF cells. Further research had found that peptides could inhibit NF-κB signaling pathway and reduced oxidative stress damage.

2.2.3 PI3K/Akt/mTOR signaling pathway

Active peptides could also alleviate oxidative stress damage by regulating PI3K/Akt/mTOR signaling pathway. *Juglans mandshurica* Maxim derived peptides (TWLPPR, YVLLPSPK and KVPPLY) could protect PC12 cells and regulated oxidative stress damage[43]. These peptides significantly increased GSH-Px activity and adenosine-triphosphate (ATP) content while prevented PC12 cells from producing ROS. Meanwhile, they regulated autophagy by modulating Akt/mTOR signaling pathway in the amyloid beta-peptide 25-35 ($A\beta_{25-35}$)-induced oxidative stress model.

2.2.4 MAPK signaling pathway

Cellular oxidative stress can regulate MAPK signaling pathway, which is closely related to cellular inflammatory response. Many publications indicate that active peptides can regulate MAPK signaling pathway. Qian et al.[44] isolated two peptides (SHP-1 and SHP-2) from the hippocampus and found that peptides treatment could alleviate ethanol-caused oxidative stress. Peptides decreased extracellular regulated protein kinases (ERK) and p38 expression and inhibited MAPK signaling pathway, while peptides could increase the levels of antioxidant-related proteins (such as SOD, GSH, Nrf2 and HO-1). They could also prevent p65 from moving into the nucleus. The result indicates that hippocampus peptides ameliorate oxidative stress damage by regulating MAPK, Nrf2 and NF-κB signaling pathways. Deng et al.[45] found that peptide DR8 (DHNNPQIR) could exert antioxidant properties by regulating MAPK pathway to alleviate renal fibrosis, which mostly accomplished by blocking the activation of ERK, p38MAPK.

Oxidative stress involves a very complex process involving multiple signaling pathways. In recent years, many peptides have been used to reduce oxidative stress-induced damage and they can regulate different signaling pathways. Further research found that Nrf2 signaling pathway is in the key position and exerts main anti-oxidation effect, meanwhile, there is crosstalk between Nrf2 signaling pathway and other anti-oxidation pathways (Fig. 1). Martin et al.[46] found that Nrf2 is the downstream target of PI3K/Akt signaling pathway and blocking PI3K activation can inhibit Nrf2 activity. In addition, PI3K/Akt signaling can regulate Nrf2 activity through GSK-3β. Xing et al.[47] found that blocking GSK-3β phosphorylation could reduce H_2O_2-induced oxidative damage *via* the activation of Nrf2-ARE signaling pathway. AMPK signaling pathway is also related to Nrf2 signaling pathway. AMPK could promote Nrf2 activation by inactivating GSK-3β[48]. AMPK also directly phosphorylated Ser558 site of Nrf2 (Ser550 in mice) and promoted the accumulation of Nrf2 in cell nucleus[49]. The interaction between Nrf2 signaling pathway and mTOR signaling pathway shows that Nrf2 can directly regulate mTOR promoter and increases mTOR expression, meanwhile, mTOR can also promotes Nrf2 nuclear translocation by inhibiting beta-transducin repeat-containing homologue protein (*β*-TrCP) expression[50-51]. p38 MAPK can phosphorylate Nrf2 and promotes the association between Nrf2 and Keap1 protein, thereby inhibiting Nrf2 nuclear translocation[52]. Song et al.[53] found that Nrf2 activation is dependent on AMPK/p38 signaling pathway. Studies have shown that Nrf2 can up-regulate HO-1 expression, which can inhibit NF-κB nuclear translocation[54]. Therefore, Nrf2 signaling pathway plays a central role in oxidative stress damage, and it is important to study peptides to reduce oxidative stress damage by targeting Nrf2 pathway.

3. Nrf2 signaling pathway

3.1 Structure of Nrf2

According to studies, Nrf2 is a key regulatory factor of the electrophile counterattack response and is the fundamental reason for electrophiles to exert detoxication enzyme activities[55]. Nrf2 has 7 functional domains: Neh1 (Nrf2-ECH homologous domain-1) to Neh7. The Neh1 domain consists of cap ‘n’ collar (CNC) and basic region-leucine zipper (bZIP), which are involved in DNA binding and dimer formation. The bZIP domain of transcription factor Nrf2 can recognize musculo-aponeurotic fibrosarcoma (Maf) protein and make Nrf2 form heterodimer with Maf protein, which can bind *cis*-regulatory element ARE and promote the expression of related-genes. The Neh2 domain is the binding region between Nrf2 and Keapl, containing 2 binding sites: ETGE motif and DLG motif. The Neh3, Neh4, and Neh5 domains regulate transactivation. Nrf2 entering the nucleus in the form of Nrf2-Maf cannot immediately initiate transcription after binding to ARE, and other helper proteins are needed. The Neh4 and Neh5 domains of Nrf2 interact with CREB (cAMP responsive element binding protein) binding protein (CBP), brahma-related gene 1 (BRG1), and mediator complex subunit 16 (MED16) to promote transcription. The COOH terminal domain Neh3 of Nrf2 interacts with chromodomain helicase DNA binding protein 6 (CHD6), which is a DNA-dependent ATPase and localizes at nuclear sites of mRNA synthesis. Neh6 is an Nrf2 domain unrelated to Keap1. It has serine residues phosphorylated by glycogen

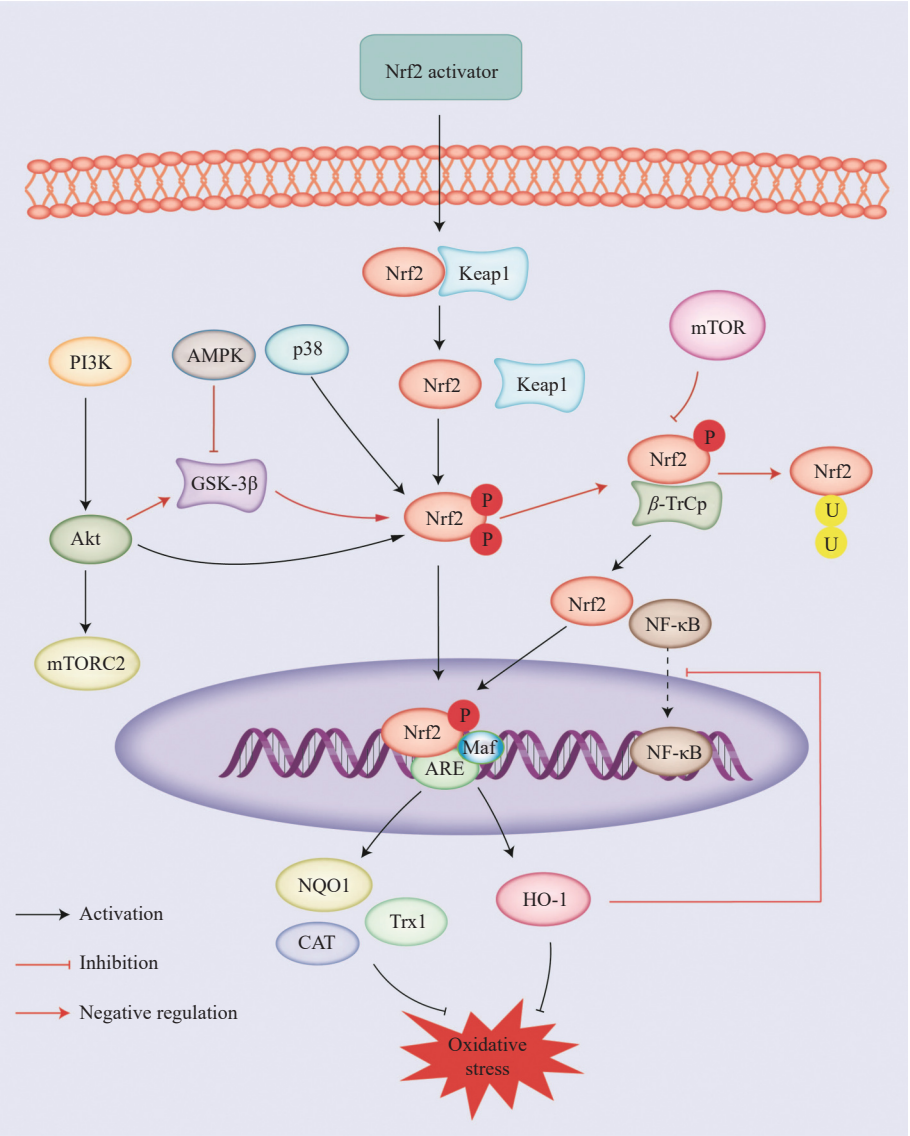

Fig. 1 Crosstalk between Nrf2 and other signaling pathways involved in oxidative stress.

synthase kinase 3 (GSK-3), which can bind to *β*-TrCP to induce Nrf2 to be ubiquitinatied and degraded. Neh7 inhibits the binding of Nrf2 and ARE by promoting the binding of Nrf2 and retinoid X receptor α (RXRα).

3.2 The activation mechanisms of Nrf2 system

Nrf2 is a redox sensitive transcription factor involved in the maintenance of cellular homeostasis and antioxidant mechanisms[56]. Currently, there are 5 main mechanisms for activating Nrf2 (Fig. 2). 1) modify the cysteine group of Keap1. The researchers found that ROS and electrophilic reagents had effects on body cells[57]. How do cells perceive these stimuli and respond to regulate Nrf2? In 1999, a protein Keap1 was discovered and it is a biosensor that allows cells to sense electrophilic reagents and ROS; meanwhile Keap1 also can regulates Nrf2 activity[58]. Keap1 binds with *β*-actin to anchor in the cytoplasm. It contains five domains, namely the N-terminal region (NTR), BTB (also named poxvirus and zinc finger (POZ)) domain, intervening region (IVR, also named BACK domain), double glycine repeat (DGR, Kelch) domain and C-terminal region (CTR). The DGR region is the binding region of Keap1 and Nrf2, and the binding site of actin in cytoplasm. The BTB region is associated with the Keap1 homodimer. The IVR region is rich in cysteine content and serves as the functional gate region of the entire protein, with cysteine residues C273 and C288 playing a key role in inhibiting Nrf2 nuclear accumulation[59]. The active residues in the IVR region (C257, C272, C288, and C297) are the reason why Keap1 become a sensor[60]. Under non-reactive conditions, Keap1 binds to cullin 3 (Cul3) through its BTB and IVR regions, while Keapl's DGR and CTR regions bind to Nrf2, thus connecting Nrf2 to the E3 complex, transferring ubiquitin from E3 to the lysine residue of Nrf2 (located between the ETGE motif and DLG motif), and the ubiquitinated Nrf2 is rapidly degraded[61-62]. Thus, under the redox balance, cells can synthesize Nrf2 but are degraded. Under oxidative stress conditions, electrophilic Nrf2 inducers modify the cysteine residue of Keap1, resulting in a weakened affinity between the DLG motif of Neh2 and Keap1, driving Nrf2 to dissociate from Keap1. Free Nrf2 enters the nucleus from the cytoplasm, forms a heterodimer with Maf protein,

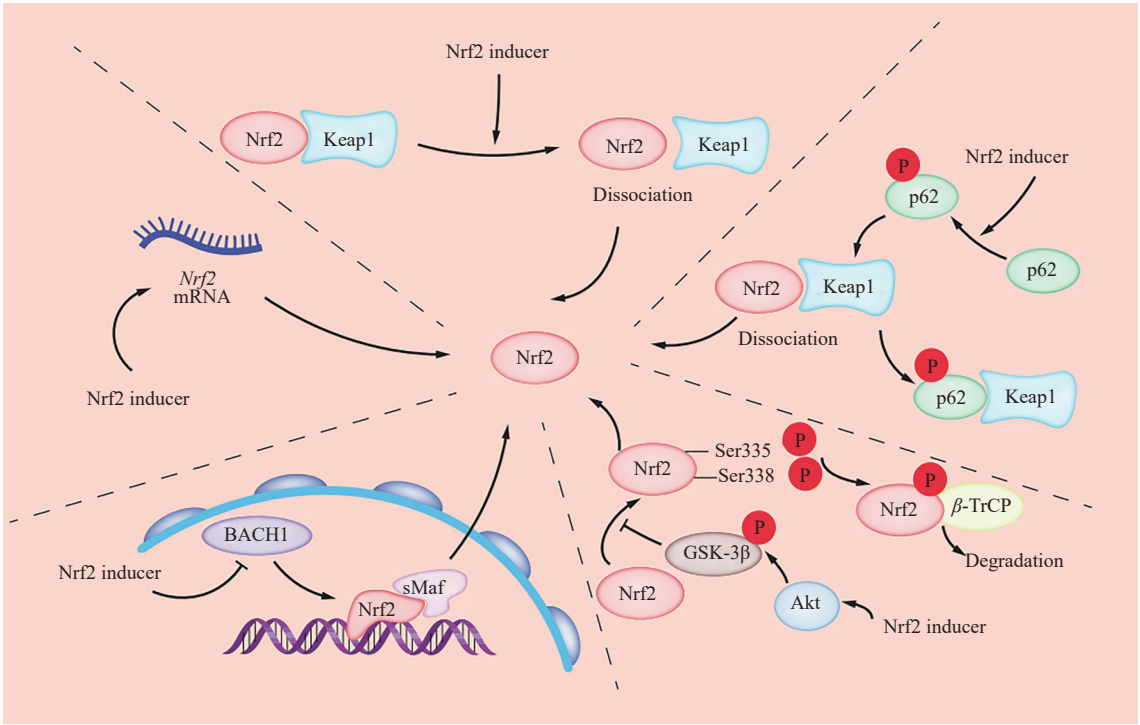

Fig. 2 Pathways and mechanisms of Nrf2 signal pathway activation.

binds to ARE, and activates antioxidant genes expression[63]. For example, Nrf2 inducers *tert* butylhydroquinone (tBHQ) and itaconic acid are electrophilic can bind to cysteine residues in Keap1, which cause Nrf2 to dissociate with Keap1 and enter the nucleus to exert antioxidant effects[64-65]. 2) The binding of *β*-TrCP and Nrf2 is inhibited. Research has shown that in addition to being regulated by keap1, Nrf2 can also be activated by PI3K-Akt system[66], which is mainly related to glycogen synthase kinase 3 beta (GSK-3β). Under normal circumstances, GSK-3β phosphorylates Nrf2 promotes *β*-TrCP binds to phosphorylated Nrf2, causing ubiquitination and degradation of Nrf2. Under oxidative stress conditions, Akt can promote GSK-3β phosphorylation, thereby inhibiting Nrf2 phosphorylation and damaging *β*-TrCP combining with Nrf2, enabling Nrf2 to smoothly translocation and bind ARE, activating antioxidant genes transcription. For example, flurbiprofen promotes GSK-3β phosphorylation by activating Akt phosphorylation, increases the level of Nrf2 in the nucleus and protects rats from glutamate excitotoxicity damage[67]. The degradation of Nrf2 in the body mainly occurs through two pathways: Nrf2 binds to Keap1 and is ubiquitinated and degraded by Cul3, Nrf2 is also related to *β*-TrCP binding is ubiquitinated and degraded by Cul1. Peptides can activate Nrf2 and exert their biological functions by regulating these two degradation pathways. For example, peptide BP5 could alleviate lipopolysaccharide (LPS)-brought about oxidative stress and inflammation through promoting Nrf2 expression, but with the knockout of Nrf2, the anti-inflammatory effect of BP5 disappeared[68]. The researchers explored the potential mechanisms through further experiments. They found that BP5 could inhibit the binding of Keap1 and Nrf2, enabled the separation of Nrf2 from Keap1 and promoted the entry of Nrf2 into the nucleus. BP5 exert the effect of anti-oxidative damage by regulating the expression of oxygen stress-related genes. Furthermore, BP5 activated Akt and GSK-3β phosphorylation. This indicated that BP5 alleviated oxidative stress and inflammatory damage by dual regulation of Nrf2 through Keap1 and Akt/GSK-3β. 3) Keap1 is modulated by p62. The Keap1-Nrf2 system is not only related to the PI3K-Akt signaling pathway, but also interacts with autophagy[69-70]. Nrf2 activator activates the phosphorylation of p62 by activating TANK-binding kinase 1 (TBK1) and mTOR complex 1 (mTORC1) complex, and the phosphorylated p62 binds to Keap1 to degrade Keap1 through autophagy pathway, thus dissociating Nrf2 and allowing it to enter the nucleus and play a role[71]. p62 is a key autophagy receptor. The mechanism by which p62 competes with Nrf2 and binds Keap1 to activate Nrf2 pathway has been studied in detail. The STGE motif in the p62 killer cell immunoglobulin-like receptors (KIR) domain is phosphorylated by the mTORC1 complex. The phosphorylated STGE motif has a higher affinity for Keap1 than the DLG motif in Nrf2 Neh2, but a lower than ETGE motif. Therefore, Keap1 binds to p62 more easily than Nrf2 and Nrf2 is dissociated from Keap1, which is conducive to the stability of Nrf2. Natural products can reduce oxidative stress damage by regulating p62-Nrf2 signaling pathway. For example, Ginseng root extract treatment activated Akt-mTOR signaling pathway, promoted phosphorylation of p62 and autophagy, which in turn activated Nrf2 nuclear translocation, alleviated ROS production and mitochondrial dysfunction in LPS-induced RAW264.7 cells. 4) Inhibit BTB domain and CNC homolog 1 (BACH1). BACH1 is a transcription suppressor, which is closely related to ROS, heme homeostasis, etc. [72]. BACH1 has 2 domains, including the BTB/POZ and bZip domains. The BTB/POZ domain is involved in protein interaction. The bZip domain can bind to DNA and can bind to small Maf proteins to form heterodimers[73]. In the nucleus, BACH1-Maf heterodimer can reduce HO-1 and NQO1 expression, thus participating in redox regulation[74-75]. The expression of antioxidant proteins (such as NQO1 and HO-1) is induced when Nrf2 from the cytoplasm reaches the nucleus and forms heterodimers with the Maf protein in the presence of ARE, which is a

key point for Nrf2 to play the antioxidant role. BACH1 in the nucleus competes with Maf protein to form heterodimers, which makes Nrf2 unable to bind to Maf protein and AREs. Therefore, inhibiting BACH1 or promoting its degradation is a key step in the activation of Nrf2. In bleomycin-induced pulmonary fibrosis mice, pirfenidone inhibited BACH1 expression and promoted the expression of Nrf2 and HO-1 to reduce lung injury[76]. 5) Regulate *de novo* synthesis of Nrf2. Nrf2 is continuously synthesized in the body and then degraded. To prevent degradation of Nrf2 by inactivated Keap1, some activators promote *de novo* synthesis of Nrf2. For example, antioxidants MnTMPyP could enhance the synthesis of Nrf2 under oxidative stress conditions, thus enhancing the protective effect on cells[77]. In summary, the activation of Nrf2 is mainly based on the above five modes, and there may be other activation modes to be further discovered.

4. Peptides alleviate oxidative stress-related diseases *via* Nrf2 pathway

Oxidative stress-related diseases are currently a major problem and millions of people worldwide are dying each year from such diseases, such as ischemia reperfusion injury, aging, inflammation and liver injury kidney injury. Peptides are part of sequences protein, which can provide health benefits in addition to basic nutritional benefits. There is evidence that bioactive peptides alleviate oxidative stress-related diseases by regulating Nrf2 signaling pathways (Fig. 3 and Table 1). In the Table 1, bioactive peptides targeting Nrf2 pathway to regulate oxidative stress-related diseases and the amino acid sequence are listed. The molecular weight of peptides can be easily calculated according to the amino acid sequence.

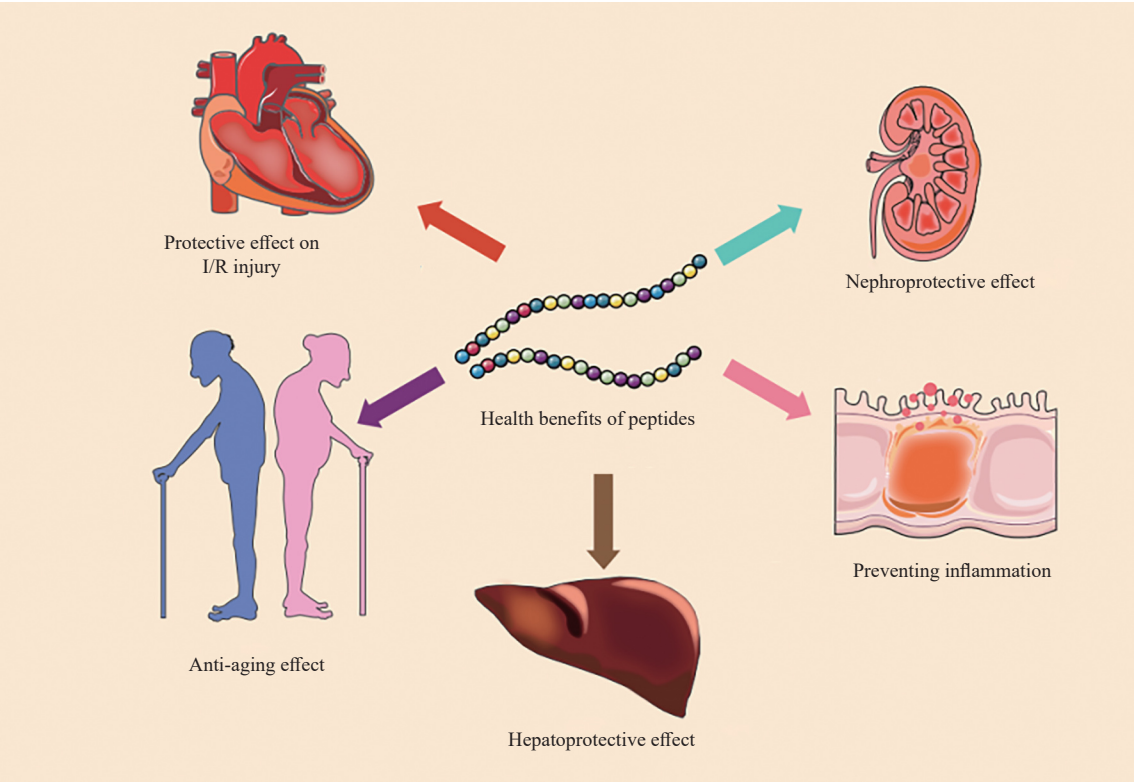

Fig. 3 Health benefits of bioactive peptides targeting Nrf2 signal pathway.

Table 1
List of bioactive peptides in this review for the management of oxidative stress-related diseases.

Source of peptide	Peptide name and sequences	Study model	Mechanisms	Effect	Reference
China peptides	ND-13, YGRKKRRKGAEEMETVIP VD	*In vitro* (oxygen glucose deprivation/ reoxygenation (OGD/R) HAPI cell) and *in vivo* (middle cerebral artery occlusion-reperfusion (MCAO/R) rat)	Bax, Cleaved-Caspase-3, total Caspase-3 ↓ Bcl-2, Nrf2, Notch receptors (NICD), Notch1, hairy and enhancer of split 1 (Hes1) ↑	Against I/R injury	[86]
Odorous frog species *Odorrana livida*	OL-FS13, FSLLLTWWRRRVC	*In vitro* (OGD/R PC12 cell) and *in vivo* (cerebral I/R rats)	SOD, CAT ↑ MDA, lactate dehydrogenase (LDH) ↓ Nrf2 (nucleus), HO-1, Bcl-2 ↑ Bax, Cleaved-Caspase-3/Caspase-3, inositol requiring enzyme 1α (IRE1α), TNF receptor associated factor 2 (TRAF2), p-JNK/JNK ↓	Against I/R injury	[88]
Chemical synthesis	APNp, LQVYGDGDHNGLYADN VN	*In vitro* (OGD/R primary astrocytes) and *in vivo* (transient middle cerebral artery occlusion (tMCAO) C57BL/6J mice)	Bax, Cleaved-Caspase-3 ↓ MDA ↓ TXNIP, NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), Caspase-1, p20, IL-1β, IL-18 ↓ Nrf2, Bcl-2 ↑ SOD, GSH-Px ↑ Trx, p-AMPK/AMPK, p-GSK-3β/GSK-3β ↑	Against I/R injury	[89]

Table 1 (*Continued*)

Source of peptide	Peptide name and sequences	Study model	Mechanisms	Effect	Reference
Chinese pond turtle	CPTP	*In vitro* (DPPH, ABTS cation, and hydroxyl radical) and *in vivo* (*Drosophila melanogaster*)	Inhibit DPPH, ABTS cation and hydroxyl radical (half maximal inhibitory concentration (IC_{50}) values of 3.31, 1.93, and 9.52 mg/mL) SOD, GSH-Px ↑ MDA, protein carbonyl (PCO) ↓ *Keap1* ↓ *CncC*, *HO-1*, *Gclc* ↑	Anti-aging	[96]
Rice bran	KF-8, KHNRGDEF	*In vitro* (H_2O_2-treated NIH/3T3 cells) and *in vivo* (*D*-gal-induced aging mice)	SOD, GSH ↑ ROS, MDA, 8-hydroxy-2′-deoxyguanosine (8-OHdG) ↓ SOD3, glutathione peroxidase 1 (Gpx1) ↑ Keap1 ↓ Nrf2, HO-1, Bcl-2 ↑ Toll-like receptor 4 (TLR4), Myeloid differentiation primary response 88 (MyD88), p-p65, IκB, TNF-α, Bax, p-p38, Cleaved-Caspase-3 and poly ADP-ribose polymerase (PARP) ↓	Anti-aging	[97]
Synthesis	MOTS-c, MRWQEMGYIFYPRKLR	*In vivo* (*D*-gal-induced aging mice)	*Foxo1*, *Sirt1*, *Nrf2* ↑ *Fasn* ↓ *Rage*, *Scd1*, *Pgc*, *IL-6* ↓	Anti-aging	[100]
Buffalo milk casein	Pep, VLPVPQK	*In vitro* (primary fibroblasts)	MDA, ROS, NO ↓ SOD, CAT, GSH ↑ TNF-α, IL-6 ↓ Bax ↓ Nrf2 ↑ NF-κB, p-p38 ↓	Anti-aging	[101]
Buffalo milk	Pep, VLPVPQK	*In vitro* (skin fibroblast cells)	*Nrf2*, *HO-1*, *Bcl-2* ↑ *Keap1*, *Bax* ↓	Anti-aging	[102]
Walnut protein	LPF	*In vivo* (dextran sulfate sodium (DSS)-induced colitis in mice)	IL-6, TNF-α ↓ Lachnospiraceae, Ruminococcaceae ↑	Anti-inflammation	[104]
Milk	K-8-K, KVLPVPEK	*In vitro* (Caco-2 cells)	MDA ↓ Nrf2, TrxR1, SOD1, Trx1, NQO1, GR ↑	Antioxidant	[111]
Fermented soy	S-10-S, SLVNNDDRDS	*In vitro* (Caco-2 cells)	Nrf2, NQO1, GR, TrxR1 ↑	Antioxidant	[112]
Synthesis	K-8-K, KVLPVPEK S-10-S, SLVNNDDRDS	*In vitro* (Caco-2 cells)	Nrf2, HO-1 ↑ NF-κB, IL-1β, IL-6, TNF-α ↓	Anti-inflammation	[113]
Walnut	WEKPPVSH	*In vitro* (LPS-activated BV-2 microglia)	NO, ROS ↓ SOD, CAT ↑ TNF-α, IL-1β, IL-6 ↓ Nrf2, HO-1 ↑ inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), p-IκB/IκB, p-p65/p65, p-p38/p38 ↓	Anti-inflammation	[114]
Korean traditional fermented Kimchi	YD1, GPNG	*In vitro* (LPS-induced RAW 264.7 cells) and *in vivo* (CA inflammatory mouse model)	NO, iNOS, COX-2, prostaglandin E2 (PGE2), TNF-α, IL-1β, IL-6, TLR4, MyD88, interleukin-1 receptor-associated kinase 4 (IRAK4), p-IκBα, NF-κB (nucleus), p-Akt ↓ Nrf2 (nucleus), HO-1, NQO1 ↑ ROS ↓	Anti-inflammation and antioxidant	[115]
Red shrimp (*Solenocera crassicornis*) head	SCHPs-F1, PVERK	*In vivo* (male ICR mice)	CAT, total antioxidant capacity (T-AOC), GSH-Px, SOD ↑ Nrf2, glutamate cysteine ligase modifier subunit (GCLM), HO-1, NQO1 ↑ NF-κB, IL-1β, IL-6, interferon-γ (IFN-γ), TNF-α ↓ AST, ALT, alkaline phosphatase (AKP) ↓	Hepatoprotection	[125]
Acaudina molpadioides	AMP	*In vitro* (H_2O_2-induced RAW264.7 cells) and *in vivo* (CCl_4-induced mice)	MDA, AST, ALT ↓ SOD, GSH-Px, CAT ↑ Nrf2, HO-1, NQO1 ↑ Keap1 ↓ p-ERK, p-JNK, p-p38 ↓ p-PI3K, p-Akt ↑	Hepatoprotection	[127]
Krill protein hydrolysates	KPF (peptide fraction)	*In vivo* (ethanol-induced liver damage BALB/c mice)	SOD, CAT, GPx ↑ IL-6, TNF-α ↓ Nrf2, HO-1 ↑ Bcl-2 ↑ Bax, Cleaved-Caspase-3 ↓	Hepatoprotection	[129]
Krill protein hydrolysates	P2, AMVDAIAR	*In vitro* (H_2O_2-induced human hepatocytes)	Nrf2, HO-1 ↑ SOD, CAT, GPx ↑ Bcl-2 ↑ Bax, Cleaved-Caspase-3 ↓ p-IkBα, NF-κB ↓	Hepatoprotection	[130]
Xuanwei ham	NPPKFD	*In vitro* (alcohol-treated HHL-5 human hepatocytes)	Nrf2, HO-1 ↑ CYP2E1 ↓ ROS ↓ ALT, AST, MDA ↓ SOD, GSH-Px, CAT ↑	Hepatoprotection	[131]
Oyster peptide		*In vivo* (alcohol-induced liver disease mouse)	AST, ALT, γ-glutamyl transferase (GGT), ROS, MDA ↓ TG, IL-1β, IL-6, TNF-α ↓ SOD, GSH ↑ Nrf2, HO-1, NQO1 ↑ NF-κB ↓	Hepatoprotection	[132]
Synthesis	Liraglutide	*In vivo* (high fat diet (HFD) induced male C57BL/6 mice)	Sestrin2, p-AMPK, Nrf2, HO-1, CAT, GCLM, NQO1 ↑ MDA, TNF-α ↓	Hepatoprotection	[133]

Table 1 (*Continued*)

Source of peptide	Peptide name and sequences	Study model	Mechanisms	Effect	Reference
Ankang fish skin	PSCP	*In vivo* (HFD induced kidney damage mice)	Uric acid (UA), creatinine (CRE), blood urea nitrogen (BUN) ↓ SOD, GSH-Px, CAT ↑ Nrf2, HO-1, NQO1, GCLM ↑ IL-1β, IL-6, TNF-α ↓ NLRP3, ASC, Caspase-1, Cleaved-Caspase-1 ↓	Nephroprotection	[138]
Acaudina molpadioides	Amp	*In vivo* (CCl_4 induced kidney damage mice)	CRE, BUN ↓ CAT, SOD ↑ Nrf2, HO-1, p-PI3K, p-Akt ↑ Keap1 ↓ NF-κB, TNF-α, IL-6, IL-1β ↓	Nephroprotective	[139]
Sea cucumber	SCP	*In vivo* (male ICR mice)	Lactic acid (LA), BUN, hyperammonaemia (NH_3), MDA, LDH ↓ SOD, GSH-Px ↑ Nrf2, HO-1 ↑ Keap1 ↓ p-AMPK/AMPK, peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), nuclear respiratory factor 1 (NRF1), mitochondrial transcription factor A (TFAM), cytochrome c oxidase IV (COX IV) ↑ ATP, Ca^{2+}-Mg^{2+}-ATPase, Na^{+}-K^{+}-ATPase ↑	Anti-fatigue	[140]
Soft-shelled turtle	STP	*In vivo* (male ICR mice)	LA, BUN, LDH, creatine kinase (CK), MDA ↓ SOD, GSH-Px ↑ Keap1 ↓ Nrf2, HO-1 ↑	Anti-fatigue	[141]
Round scad	WCPFSRSF	*In vitro* (glutamate-induced SH-SY5Y cells) and *in vivo* (sleep deprivation mice)	Nrf2, HO-1, NQO1 ↑ p-Akt ↑ NF-κB ↓ SOD, GSH-Px ↑ ROS, LDH, MDA ↓	Neuroprotection	[142]
Cicadidae periostracum	Suntamide A	*In vitro* (rotenone-induced SH-SY5Y cells)	ROS ↓ SOD, GSH ↑ Nrf2, p-Akt, p-ERK ↑	Neuroprotection	[143]
Skin collagen hydrolysate of *Takifugu bimaculatus*	FNLRMQ	*In vitro* (Ang II-induced HUVEC cells)	Bax, Caspase-3, cytochrome c ↓ Bcl-2 ↑ ROS, MDA, LDH ↓ T-AOC ↑ Nrf2 (nucleus), HO-1 ↑ Keap1 ↓ p-eNOS, PI3K, p-Akt ↑	Protect endothelial injury	[144]

4.1 Protection against I/R injury

For ischemic diseases such as myocardial infarction and stroke, timely blood reperfusion will reduce infarct size and injury. During the process of blood reperfusion, a large amount of ROS is produced and results in I/R injury, exploring new clues to prevent and reduce I/R injury has become an urgent need to solve the problem.

Nrf2 signaling pathway plays an indispensable role in alleviating I/R injury. I/R injuries of the brain, heart and kidneys are typical example of oxidative tissue damage. Exogenous and endogenous Nrf2 inducers can reduce tissue damage after I/R[78-81]. Noise-induced hearing loss is also considered an I/R injury. Similarly, Nrf2 inducer pretreatment can also effectively prevent hearing damage[82]. Peptides can activate the Nrf2 pathway and provide protection against heart damage by regulating in anti-oxidation, anti-inflammation and autophagy.

High levels of ROS induce apoptosis in I/R through DNA damage, activation of apoptosis signaling pathways and stabilization of hypoxia-inducible factors[83-85]. ROS is one of the important mechanisms involved in the occurrence of I/R injury. In cerebral I/R injury, the DJ-1-based polypeptide ND-13 functions as a neuroprotective agent by upregulating Nrf2 expression and inhibiting cell apoptosis[86].

ERS can lead to apoptosis of stress cells and is a key participant in I/R injury[87]. Protein kinase like endoplasmic reticulum kinase (PERK) is the pressure sensor of ER, which can prevent abnormal protein accumulation. Nrf2 translocation may participate in the ERS-PERK signaling pathway, thereby alleviating I/R injury. Liu et al.[88] showed that the neuroprotective peptide OL-FS13 could inhibit ERS and reduced the infarct size of I/R injury rats, which were mediated by Nrf2 signal pathway.

The inflammatory response after I/R is an important cause of cell injury. Therefore, reducing inflammatory response after reperfusion is crucial for the treatment of diseases. Adiponectin peptide (APNp) promoted nuclear translocation of Nrf2 by increasing AMPK, GSK-3β and thioredoxin-1 (Trx1) expression, and alleviated oxidative stress damage after cerebral I/R injury[89]. In addition, APNp further reduced thioredoxin-interacting protein (TXNIP) levels by activating Nrf2, inhibited the activation of nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome during cerebral I/R injury. These results indicated that APNp could alleviate brain I/R injury through antioxidant, anti-inflammatory, and anti-apoptotic effects. Generally speaking, peptides can regulate oxidative stress, ERS, apoptosis, and inflammation by regulating Nrf2 signaling pathway, and alleviate I/R injury. However, the current application of bioactive peptides in this field is limited and needs further improvement.

4.2 Anti-aging function

Aging is a biological process that results in damage to cellular structure and function[90]. Oxidative stress-induced damage accumulation is one of the most widely accepted theories to explain aging[91]. Inhibition of the Nrf2 pathway reduces anti-stress capacity and lifespan in aging mice[92-93]. The Nrf2 pathway is closely associated with senescence, and Nrf2 can inhibit cell aging by regulating antioxidant defense systems and is a key regulator promoting longevity[94-95]. Therefore, it is important to search for new drugs that regulate Nrf2 pathway to delay aging and treat age-related diseases.

This review focuses on the progress of Nrf2 regulatory peptides and provides new clues for the development of anti-aging drugs.

Many studies have reported that peptides can exert anti-aging functions by regulating the Nrf2 signaling pathway. Peptides from Chinese pond turtle (CPTP) had anti-aging effects, manifested as female fruit flies that received CPTP treatment lived longer without limiting food intake, and enhanced their resistance to oxidative stress (30% H_2O_2) and heat stress (37 °C)[96]. CPTP therapy prolonged the lifespan of fruit flies by increasing SOD and GSH-Px activities and reduced peroxidation products MDA levels. According to additional experimental findings, CPTP decreased *Keap1* expression while increased the expression of *CncC* (a *Drosophila* homolog of mammalian *Nrf2*), as well as its downstream target genes *HO-1* and *Gclc*. Taken together, CPTP activates the Nrf2 signaling pathway and has anti-aging effects.

Long term use of *D*-galactose (*D*-gal) will lead to the accumulation of galactitol in the body, which will lead to oxidative stress, aging and organ damage. *D*-gal induced aging is a reliable experimental model to evaluate the efficacy of new compounds to treat aging related illness. The active peptide KF-8 inhibited oxidative stress-related organ damage in *D*-gal induced aging mice by regulating Nrf2 and NF-κB pathways[97]. Peptide MOTS-c has been established through research to control cell metabolism and inflammation[98-99]. In the *D*-gal-induced aging mouse model, MOTS-c treatment showed that the collagen fiber content in the dermis was increased *via* raising *Nrf2* and reduced the expression of interleukin 6 (*IL-6*)[100]. The anti-aging activity of MOTS-c may be achieved by reducing inflammation.

Long term passage of primary fibroblasts can reflect age-related characteristics. Therefore, it is a good natural tool for describing the molecular mechanisms of cellular aging. Kumar and his colleagues[101] revealed the anti-aging effect of an active peptide (VLPVPQK) isolated from buffalo milk casein using primary fibroblasts. VLPVPQK treatment could enhance the migration of aging fibroblast cell migration and reduced the contents of MDA, ROS, NO, TNF-α and IL-6 in aging fibroblast. In addition, the peptide also reduced the expression of Bax and senescence-associated *β*-galactosidase. The anti-aging mechanism of active peptide (VLPVPQK) was through activating Nrf2 mediated antioxidant defense system and inhibiting NF-κB/p38 MAPK signaling pathway. Starvation conditions can lead to cell damage or ROS-producing ulcers. Kumar et al.[102] analyzed the anti-apoptotic effect of peptides by establishing *in vitro* models of serum starvation skin fibroblasts. VLPVPQK could protect serum hungry cells by promoting *Nrf2*, *HO-1*, and *Bcl-2* expressions. Additionally, *Keap1* and *Bax* expressions could be suppressed by VLPVPQK, thus inhibiting cell apoptosis. The future application of bioactive peptides as anti-aging agents will be interesting.

4.3 Protection against inflammatory damage

Inflammation is a complex physiological response that protects the body from external toxins and stimuli. However, excessive inflammatory response is harmful to the body. Inflammatory factors can attack the body's own tissues and damage tissues and organs. Inflammation is an important factor in the development of chronic diseases, and is responsible for most deaths from diseases such as AD, cardiovascular disease, and cancer. Most traditional anti-inflammatory drugs have side effects, and bioactive peptides can serve as substitutes for traditional anti-inflammatory drugs, with mild and effective therapeutic effects on various chronic diseases[103]. Inducing factors such as LPS, dextran sodium sulfate (DSS) and other toxic substances will stimulate the inflammatory pathway, lead to the release of inflammatory factors, and eventually cause inflammation. Studies have shown that peptides can alleviate inflammation, such as peptide LPF derived from the walnut protein could reduce inflammatory response in the DSS-induced colitis of mice. Peptide LPF treatment reduced IL-6 and TNF-α, reduced cell apoptosis, increased splenic regulatory T cells, regulated intestinal microbiota, could help alleviate colitis symptoms and recovery[104]. Therefore, the role of peptides in inflammatory diseases has attracted people's attention.

In addition to enhancing the antioxidant capacity of cells, Nrf2 can also stimulate strong anti-inflammatory activity. Nrf2-deficient mice are highly sensitive to LPS-induced acute inflammation, and activation of Nrf2 effectively prevents LPS-stimulated death[105]. In another study, Nrf2 protected the airway epithelium of asthma mice by reducing allergic inflammation and airway hyperresponsiveness[106]. The molecular mechanism of Nrf2 anti-inflammatory may have two aspects. Firstly, Nrf2 exerts anti-inflammatory effect by directly antagonizing inflammatory factors like IL-6 and interleukin-1β (IL-1β). However, the exact mechanism by which Nrf2 prevents inflammatory factors is still unknown. Secondly, Nrf2 adversely controls the NF-κB signaling pathway[107], thereby exerting anti-inflammatory effects. It has been reported that peptide can protect cells or tissues from inflammatory damage through NF-κB and Nrf2-ARE pathways[108-110]. Peptides K-8-K and S-10-S could activate Nrf2 and NQO1 expression was first discovered by Tonolo et al.[111-112]. To study the interaction between Nrf2 and NF-κB, Tonolo et al.[113] found that the Nrf2-activating peptides K-8-K and S-10-S could inhibit NF-κB pathway and reduced exhibit inflammatory response. However, Tonolo et al.[113] believes that the interaction between Nrf2 and NF-κB needs further study. Walnut-derived peptide WEKPPVSH protected BV-2 microglia from LPS-induced oxidative stress and inflammatory damage by regulating Nrf2/HO-1 and NF-κB/p38/MAPK signaling pathway[114]. Rahman et al.[115] isolated peptide YD1 from Korean traditional fermented Kimchi, and found that intervention with peptide YD1 could reduce LPS-induced macrophage inflammation and carrageenan (CA)-induced foot edema in mice. Its mechanism of action is mainly related to the inhibition of oxidative stress and inflammation, and shows a crosstalk mechanism involving Nrf2 antioxidant pathway and NF-κB inflammatory pathway.

Peptide YD1 can activate Nrf2 signaling, thereby promoting its downstream proteins HO-1 and NQO1, which can inhibit the phosphorylation of inhibitor of NF-κB (IκB) and subsequent activation of NF-κB and subsequently proinflammatory cytokine production, play an anti-inflammatory role. These results suggest that peptides can play an anti-inflammatory role by regulating Nrf2 signaling.

4.4 Hepatoprotective function

Oxidative stress is an important cause of liver disease[116]. The main reason for that is excessive ROS can cause lipid peroxidation, inflammation, and fibrogenesis, followed by liver fat accumulation[117]. In addition, ROS accumulation lead to liver insulin resistance, ultimately leading to liver cell apoptosis[118]. Therefore, reducing oxidative stress in liver injury is of great significance for liver protection. Studies has shown that Nrf2 signaling pathway is a crucial

pathway for alleviating liver injury by enhancing the oxidative defense system[119]. On the other hand, the pharmacological activation of Nrf2 promotes liver regeneration[120]. Therefore, Nrf2 has positive implications for hepatoprotection.

The unreasonable use of various prescription drugs and over-the-counter drugs (such as small chemical molecules, biological agents, health care products, dietary supplements, etc.) can cause drug-induced liver injury[121-124]. For example, Cyclophosphamide (CTX) is a drug for treating human malignant tumors and leukemia, but the metabolite acrolein of CTX can cause liver injury, liver necrosis, congestion in the center of liver lobule, and increase of aminotransferase. Jiang et al.[125] found through research that peptides SCHPs-F1 had protective effects on CTX caused hepatotoxicity *via* regulating Nrf2 and NF-κB signaling pathways. SCHPs-F1 mainly exerts liver protective effects through two aspects: SCHPs-F1 activates Nrf2 signaling, increases HO-1 and NQO-1 levels, restores CAT, GSH-Px, and SOD expression, and reduces oxidative stress response; In addition, SCHPs-F1 can inhibit NF-κB signaling. A well-known liver toxin, carbon tetrachloride (CCl_4), can result in liver damage, which is characterized by central necrosis of liver lobules, infiltration of inflammatory cells, changes in central fat in liver lobules, and cell death. According to research, one of the reasons why CCl_4 causes liver damage is oxidative stress[126]. A study suggested that collagen peptide from *Acadina molpadioides* (Amp) exerted antioxidant effects through Nrf2, PI3K/Akt and MAPK pathways to alleviate CCl_4 induced liver damage in mice[127]. In conclusion, peptides can reduce drug-induced liver injury by regulating Nrf2 signaling.

Long term excessive drinking can cause multiple organ dysfunctions and may lead to the occurrence of alcoholic liver disease (ALD). ALD will further develop into alcoholic steatohepatitis and eventually into hepatocellular carcinoma. Natural food ingredients have been shown to have the potential to treat ALD[128]. Park et al.[129] found that peptide fraction (KPF) from krill protein hydrolysates could protect alcohol-induced damage. KPF could lower the contents of glutamic grass transaminase, glutamic acetone transaminase and cholesterol in the ethanol-induced liver injury mice. Moreover, KPF could increase the levels of SOD, CAT and GPx in liver tissue, reduced TNF-α and IL-6 expression, and inhibited proapoptotic related proteins Bax and Caspase-3 expression, promoted Bcl-2 expression. The mechanism by which KPF exerted liver protection may be stimulated Nrf2 signaling pathway. Subsequently, their research group isolated three peptide segments from KPF[130]. After research, it was found that peptide P2 could increase SOD, CAT and GPx activities, activated Nrf2/HO-1 and ERK pathways, alleviated oxidative stress in the H_2O_2-induced hepatocytes. P2 also helped human hepatocytes resist H_2O_2-induced apoptosis *via* regulating Bcl-2/Bax and Caspase-3 expressions. The hexapeptide (NPPKFD) derived from Xuanwei ham could activate Nrf2 signaling pathway and downregulated cytochrome P450, family 2, subfamily E, polypeptide 1 (CYP2E1) expression, which could reduce ROS content and enhanced the oxidative defense system, and thereby alleviating alcohol-induced oxidative stress damage in the liver cell HHL-5[131]. Oyster peptide could activate Nrf2 pathway and promoted the expressions of HO-1 and NQO1 in the ALD mice, which reduced oxidative stress-induced damages. Meanwhile, oyster peptide also could inhibit NF-κB pathway and reduced the expressions of inflammatory factors such as TNF-α and IL-6 in the ALD mice, so the peptide can reduce inflammation-induced damages[132]. Taken together, oyster peptide can exert protective effect *via* enhancing the antioxidant capacity of body and inhibiting inflammatory response in the ALD mice.

Obesity is an important cause of nonalcoholic fatty liver disease (NAFLD) damage. Obesity can cause lipid oxidation, insulin resistance, imbalance in redox homeostasis, and subsequently lead to excessive oxidative stress, lead to mitochondrial damage, trigger liver damage and cell apoptosis. Liraglutide improves obesity-related NAFLD *via* enhancing antioxidant protein sestrin2 expression, thereby activating Nrf2/HO-1 pathway[133]. Hyperglycemia and high fat can cause liver injury in patients with diabetes. Protein hydrolysates are used to treat liver injury due to their anti-diabetes and antioxidant properties. In a study, casein hydrolysate could alleviate liver injury in streptozotocin/high-fat-induced diabetes rats *via* increasing Nrf2 expression and endogenous antioxidant enzymes activities[134].

In summary, bioactive peptides are potential functional foods that enhance liver function and health, and further research on bioactive peptides is necessary. We believe that Nrf2 is a potential target of liver protection, which will provide a new strategy for the treatment of liver injury.

4.5 Nephroprotective activity

The Nrf2-ARE pathway is regarded as an effective therapeutic approach for preventing the advancement of kidney disease, and oxidative stress is a significant contributor to severe kidney disease[135-137]. Pepsin-solubilized collagen peptide (PSCP) extracted from Ankang fish skin had a protective effect on chronic kidney injury mice fed with HFD[138]. PSCP could ameliorate the histopathology of kidney and alleviated renal injury in the HFD-induced mice. The protective mechanisms of PSCP include to increase the activities of SOD, GSH-Px and CAT, activate the Nrf2 pathway and inhibit NLRP3, thereby reducing IL-1β, IL-6 and TNF-α expression levels. Miao et al.[138] pointed out that PSCP could stop or slow down chronic kidney injury. In another research[139], peptides from Amp alleviated CCl_4 caused kidney damage by regulating Nrf2 signaling pathway. The Amp feeding dramatically raised CAT and SOD activities in comparison to the model group, and Amp increased Nrf2 expression in the kidneys. In a word, peptide alleviates kidney disease injury by regulating Nrf2 signaling, which provides data support for the application of peptide in functional foods or pharmacy.

4.6 Other protective activities

Peptides also play a positive role in other diseases through the Nrf2 signaling pathway. Two types of sea cucumber peptides (SCP) isolated from *Acadina leucoprocta* enhanced exercise performance *via* Nrf2 and AMPK signaling pathways[140]. Turtle peptide could improve the energy metabolism and oxidative stress by activating the Nrf2 signal pathway and enhanced the endurance in the exercise-induced fatigue mice[141]. In addition to anti-fatigue activity, peptide from round scad exerted neuroprotection by regulating Akt/Nrf2/NF-κB signal transduction[142]. Suntamide A, a neuroprotective cyclic peptide from *Cicadadae periodicum*, regulated Nrf2 signaling pathway to enhance antioxidant capacity and protect SH-SY5Y cells from rotenone-mediated neurotoxicity[143]. Cai et al.[144] found that the ACE inhibitory peptide FNLRmq could alleviate angiotensin II (Ang-II) induced damage in HUVECs, and this protective effect was achieved

via regulating the Nrf2 and PI3K/Akt signaling pathways. Other activities of peptides also need to be further explored.

5. Conclusions and perspectives

Targeting Nrf2 pathway by bioactive peptide provides new clue for the prevention and treatment of oxidative stress-related diseases. The molecular mechanisms include: 1) antioxidant peptides can activate Nrf2 pathway and increase the levels of SOD, GR, GSH-Px, and CAT, thereby decreasing the level of ROS and increasing the antioxidant capacity. 2) Antioxidant peptides negatively regulate NF-κB pathway by activating Nrf2, which can inhibit the expressions of inflammatory factors and alleviate oxidative stress-induced injury. 3) Antioxidant peptides regulate the expressions of apoptosis-related genes by activating Nrf2 pathway, and alleviate oxidative stress-induced injury through inhibiting apoptosis.

Although targeting Nrf2 pathway by antioxidant peptides shows broad application prospects, there are still a series of problems to be solved: 1) What is the mechanism by which peptides bind Nrf2 to promote its activation? What do these peptides have in common? 2) The stability of antioxidant peptide. If antioxidant peptides are taken orally, they may be degraded by digestive enzymes. At present, pepsin, trypsin and intestinal peptidase are mainly used to prepare antioxidant peptides which are resistant to the degradation of digestive enzymes and solve the degradation problem of some antioxidant peptides. Adding chemical groups can enhance the stability of antioxidant peptide and changing peptide into cyclic peptide also can increase their stability. In addition, the stability of antioxidant peptides in blood recycle also needs attention. The stability of antioxidant peptide is the basis of exerting its biological functions. 3) Immunogenicity of antioxidant peptides. As a macromolecule, antioxidant peptides may have immunogenicity, what amino acid residues linking together will lead to its immunogenicity, which need further study. In addition, antioxidant peptide modifications may also increase its immunogenicity. Reducing the immunogenicity of antioxidant peptide is a key problem to be solved in the clinical applications. 4) Absorption and utilization of antioxidant peptides. Antioxidant peptides belong to macromolecules, which have the problem of absorption availability. How the acidity, basicity and polarity of antioxidant peptides affect their absorption and transmembrane transport? These questions need to be further studied. In particular, when antioxidant peptides need to cross the brain, they also need to cross the blood-brain barrier (BBB). What characteristics of antioxidant peptides are required to cross BBB? 5) Highly efficient preparation of Nrf2-targeting peptides. New techniques such as bioinformatics, molecular docking and molecular dynamic simulations are used to screen Nrf2-targeted antioxidant peptides. Improving its targeting specificity and efficacy of antioxidant peptides will also need to be addressed, as well as investigate into its pharmacokinetics. In summary, previous studies on antioxidant peptides have been limited to the evaluation of peptide antioxidant ability in tube or using cell models to assess the antioxidant ability of peptides and its mechanisms. In the future, more animal models and clinical trials are needed to evaluate the antioxidant activity of pepetide and its mechanism. Our group also made some attempts in this aspect, and found that active peptide could alleviate the oxidative stress-induced kidney injury by targeting Nrf2 pathway in the rat model of myocardial I/R[17]. Unfortunately, strong clinical trials are still lacking.

Conflict of interest

The authors declare no conflicts of interest.

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