**Nrf2 participates in progression of osteoarthritis through modulating redox balance**

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**Abstract:**

Osteoarthritis (OA) is one of the most common joint degenerative diseases involved in age, obesity as well as joint trauma, and is always companied with pain, joint deformities and dysfunctions. Recent studies proved that OA is closely related to oxidative stress, which serves as the major cause of chronic inflammation in cartilage, leading to irreversible structural changes in the joint. As a transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2) plays an important role in anti-oxidation system, which regulates the expression of the cytoprotective genes to facilitate the elimination of reactive oxygen species (ROS). In this review, we have summarized the dramatic function of oxidative stress in OA pathology, established a complex regulatory network of Nrf2 in OA progression, and aimed to provide novel insight into the treatment of OA.

**Keywords:** Osteoarthritis (OA); Nuclear factor-erythroid 2-related factor (Nrf2); Oxidative Stress; Redox Balance; Chondrocyte

1. **Introduction**

As a common chronic degenerative disease, osteoarthritis (OA) is associated with an increasingly severe obesity and global population aging, which has become a comprehensive public health burden, currently affecting more than 500 million people worldwide [1]. OA is mainly characterized by pain as a primary clinical manifestation, which gradually affects the stability of joint movement and ultimately leads to the disability, thereby diminish the quality of patient’s life. To date, the risk factors that contribute to the development of OA are diverse, including senescence, trauma, obesity, as well as mechanical loading and many other factors [2]. And aging is one of the most evident risk factors for pathogenesis of OA and studies have shown the presence of a large number of senescent chondrocytes in the cartilage of hip and knee joints in OA patients [3, 4]. Notably, with the progression of aging, senescent chondrocytes gradually accumulate in OA joints and secrete a variety of senescence-associated secretory phenotype (SASP), including diverse pro-inflammatory cytokines and matrix metalloproteinases (MMPs), which can activate chronic inflammatory responses and induce oxidative stress, thus contributing to the accumulation of reactive oxygen species (ROS), as well as leading to the disturbance of the antioxidant enzymes and ROS clearing systems [5]. ROS are the toxic byproducts of aerobic metabolism, which are both essential and lethal for cell behaviors [6]. On the one hand, ROS are required for cellular signal transduction in many basic biological processes including cell growth and differentiation [7]. On the other hand, ROS can be toxic to DNA, proteins and lipids, which results in severe oxidative damage to cells and even kill them. Recent studies have proved that low-grade chronic inflammation increased ROS accumulation and leads to excessive oxidative stress, which causes abnormal cell metabolism in chondrocytes and thereby accelerating cartilage degradation [8]. Therefore, the maintenance of oxidant–antioxidant balance and redox homeostasis at OA chondrocyte is essential for OA treatment and remission.

Nuclear factor-erythroid 2-related factor (Nrf2) is widely known as the important component of anti-oxidative stress system, which is involved heavily in the cellular defense against multiple pathological stressors and maintains the homeostasis of intracellular environment. Nrf2 functions cytoprotective roles by modulating the expression of various important genes that participate in the clearance of ROS, reactive nitrogen species (RNS), and electrophiles. Specifically, Nrf2-induced antioxidation is generally achieved by enzymes that regulate the biological synthesis, utilization, and regeneration of the reduced glutathione (GSH). In the process of GSH synthesis, Nrf2 target three key enzyme genes, including glutamate-cysteine ligase catalytic (GCLC) and modulator (GCLM) subunits as well as glutathione synthetase (GSS). In addition, the redox cycling enzymes thioredoxin, thioredoxin reductase and many other glutathione S-transferases that are responsible for eliminating ROS are also the targets of Nrf2 [9]. Interestingly, Nrf2 also forms a large network of cooperating enzymes in the regulation of basal metabolic process, mainly including metabolic enzymes that participate in the pentose phosphate pathway and fatty acid metabolism, which form a potential bridge between redox and intermediary metabolism [5, 10-12].

It has been well studied that Nrf2 protein consists of seven functional domains, including Neh2, Neh4, Neh5, Neh7, Neh6, Neh1, and Neh3 from the N terminal to the C terminal. Among these functional domains, Neh2 and Neh6 affect Nrf2 stability, with the Neh2 domain responsible for Keap1-mediated Nrf2 degradation, and Neh6 participating in the negative modulation of Nrf2 via the serine-rich domain [13]. Neh3 could recruit the chromo-ATPase/helicase DNA-binding protein and Neh4 and Neh5 form the transactivation domain to recruit CREB-binding protein (CBP), which participate Nrf2-dependent transcriptional activation of genes with the ARE sequence in the promoter [14]. Neh1 domain promotes Nrf2 heterodimerization with small musculoaponeu-rotic fibrosarcoma (Maf) proteins to achieve DNA-binding [15, 16]. Additionally, Neh1 interacts with the ubiquitin-conjugating enzyme UbcM2 that enhances the transcriptional activity of endogenous of Nrf2 [17]. Neh7 domain binds and interacts with the retinoid X receptor α (RXRα), which is a repressor of Nrf2 [18]. Recent studies have proved that Nrf2 makes a crucial role in numerous common human diseases associated to oxidative stress such as type 2 diabetes and cardiovascular disease. Indeed, *Hinoi, E et al* and*Solomon, L et al* found that appropriate Nrf2 activity is also essential for differentiation and maturation of chondrocytes and cartilage metabolism [19, 20]. Additionally, Nrf2 has also been found to modulate osteoclastogenesis in bone resorption and remodeling , further demonstrating the key role of Nrf2 in bone homeostasis [21]. Therefore, we summarized the underlying mechanisms by which Nrf2 regulates oxidative stress in OA and aimed to develop novel biopharmaceuticals for OA treatment.

1. **Pathogenesis of OA**

OA, the most common chronic joint disease with rising incidence as the ageing and increasingly obese population, is a major cause of disability in the elderly and has a significant impact on socioeconomic costs [22-24].In recent years, increasingly researchers have focused on the important role of articular cartilage during OA progression. Articular cartilage, a special dense connective tissue, is composed of chondrocytes and extracellular matrix, and has avascular and alymphatic properties, which impinges on its natural ability for self-repair [22, 23]. Articular cartilage covers the load-bearing surface of bone to form a smooth joint contact surface that absorbs external mechanical pressure or stimuli to achieve painless joint movement [24].

OA is a degenerative alteration that disrupts the dynamic balance between joint tissues destruction and repair, resulting in loss of normal physiological function of the joint [25].The complex pathogenesis of this disease involves various aspects, such as increased inflammatory components, mechanical overload, and metabolic disorders, which gradually contributes to structural alterations in articular and periarticular tissues, specifically leading to apoptosis of chondrocytes and progressive degeneration of articular cartilage [22, 26-29].Chondrocytes in OA are activated by various factors that lead to cartilage degradation, such as high mechanical load, erosion of a large number of pro-inflammatory cytokines, unbalanced oxidative stress, and senescence, among others, which leads to the disruption of joint homeostasis and further induces the production of matrix-degrading enzymes, resulting in bone metabolic disorders and aberrant inflammatory osteolysis [30-32]. Initially, alterations in the function of chondrocytes in OA cause progressive cartilage disintegration and deterioration, which may be accompanied by persistent secondary inflammation [33].As cartilage degeneration progresses, osteoclast-induced bone resorption is abnormally accelerated, leading to bone cysts and sclerotic bone formation. In addition, cartilage and bone loss disrupt the homeostasis of bone matrix, triggering compensatory osteoblast-mediated bone remodeling. In advanced disease, deeper cartilage fissures are followed by over-synthesis of osteoblasts, leading to osteophyte formation at the joint margins, expansion of the calcified cartilage zone, as well as periarticular fibrosis [22, 34].

It is noteworthy that hypertrophic chondrocytes show higher synthetic activity during repair process, producing numerous pro-inflammatory mediators and stromal degradation products, which act upon the adjacent synovium to facilitate proliferation and inflammatory responses, and are accompanied by tissue hypertrophy and angiogenesis [22, 35].Insights into the disease pathophysiology highlight that mutations or errors in gene expression of matrix molecules and certain factors modulating matrix components synthesis are able to lead to chondrocyte hypertrophy and dysfunction, which results in chondrodysplasia at a relatively early age [34].Besides, chondrocytes located near load-bearing regions are more likely to acquire this altered phenotype, and OA susceptibility also increases with aging. In the middle and late phases of OA, diverse cell types (including chondrocytes, osteoclasts, osteoblasts, and immune cells, etc.) may be involved in the pathogenesis, and all of them may exhibit abnormal gene expression and disruption of oxido-antioxidant balance, ultimately leading to OA predisposing in the elderly population [5].

1. **Dramatic role of oxidative stress in OA pathology**

Over the past few decades, numerous researches have demonstrated that oxidative stress plays an integral role in the pathogenesis of different age-related diseases, including cardiovascular, bone, renal, as well as neurodegenerative diseases [36]. Furthermore, increased oxidative stress and decreased mitochondrial antioxidant ability affect physiological cellular signal transduction pathways, which may contribute to senescence through progressive loss of cellular integrity and disruption of tissue homeostasis [37, 38]. Regarding OA, there is a growing consensus that oxidative stress is a driver of an imbalance in catabolic and anabolic signals in cartilage, which gradually induces degradation of bone matrix with the progression of the disease, further resulting in aberrant inflammatory osteolysis [39].

ROS, composed of superoxide anion (O2**·**−), hydrogen peroxide (H2O2), and hydroxyl radical (OH), among others, are by-products of normal cell metabolism, which are generated in electron transport chain reactions and primarily responsible for transferring electrons to molecular oxygen in the mitochondria [40].This process is limited by the oxidoreductase p66Shc, which reacts to exogenous signals transmitted by growth factor deprivation, oxidative stress, and ultraviolet radiation, thus translocating to mitochondria [40, 41].ROS in mitochondria can cause oxidative stress and is a predominant regulator of cellular senescence, inducing multiple genes to facilitate mitochondrial dysfunction, swelling and apoptosis associated with aging, as well as trigger senescence or dedifferentiation of chondrocytes [42].Additionally, a portion of ROS are produced by non-mitochondrial pathways, namely NADPH oxidase (NOX) or dual oxidase (DUOX) exist in discrete regions of plasma or endosomal membranes. And NOX enzymes can regulate downstream signals for cell activation, differentiation, proliferation, and apoptosis in healthy cells, meanwhile are responsible for limiting H2O2 to specific cellular microdomains and preventing its diffusion in the cytoplasm, thereby blocking abnormal signaling [43, 44]. It has been reported that ROS (such as H2O2 and O2·−) and RNS (including ·NO and peroxynitrite (ONOO-)) play a major role in regulating chondrocyte function, disrupting cartilage homeostasis as well as inducing the progression of osteoarthritis [45-47].

In chondrocytes, osteoblasts, and osteoclasts of OA, anomalous ROS signaling is often accompanied by the spatiotemporal progression of damage from articular surface to the subchondral bone. *Yudoh, K et al* have found that the antioxidant capacity in the degenerated cartilage region in OA patients was dramatically lower than that of the intact cartilage region, thus indicating that oxidative damage in degenerated cartilage was increased compared with normal cartilage [48]. Intriguingly, based on in vitro experiments, H2O2-cultivated chondrocytes have possessed shorter telomere length. Moreover, studies have shown that when OA cartilage tissue is treated with H2O2, glycosaminoglycans (GAGs), a long linear polysaccharide that can attach to the articular surface with lubrication and protection effects, gradually reduced in a time-dependent way. Nevertheless, the use of antioxidants has been confirmed to reverse above effects, restrain GAGs loss and sustain telomere length [49].In fact, ROS exerts a significant effect in the dynamic balance of osteoclast-mediated bone resorption and osteoblast-mediated bone remodeling under physiological conditions, which is conducive to maintain bone integrity. However, abnormal levels of ROS may negatively regulate mitochondrial function and lead to changes in signal transduction pathways and gene expression, which can induce chondrocytes apoptosis and senescence, ultimately contributing to cartilage degeneration, as well as alterations in subchondral bone and bone remodeling processes [5].

It is well known that ROS production and clearance in cells are in a state of dynamic balance under physiological conditions, thus maintaining the homeostasis of the internal environment in the cytoplasm. One caveat is that there are some scavenging systems that can be used to detoxify ROS, consisting of catalase, SOD, as well as GSH peroxidase and reductase, etc [45]. As a dimeric cytosolic enzyme, SOD1 combines copper and zinc (Cu/Zn-SOD), while SOD2 is a mitochondrial homotetramer binding one manganese ion per subunit (Mn-SOD). Both enzymes are responsible for the conversion of superoxide to H2O2 and diatomic oxygen. And composed of a tetrameric protein, catalase is able to convert H2O2 to H2O and gaseous O2. Moreover, in the cytosol, the GSH peroxidase and GSH reductase system maintains the reducing environment in cells. When ROS production escapes the antioxidant systems and mechanisms, the cells are negatively affected by oxidative stress and turn out sensitive to the activation of apoptotic pathways. ROS-mediated damage can be often reversed by repair, replacement, degradation, or sequestration of the damaged macromolecules, but in some cases the stress can be sustained, driving mitochondrial and cell death or mutagenesis [40, 41]. The biological effects of ROS in all cell types are due to hyperperoxidation, protein carbonylation, direct DNA damage, telomere shortening, epigenetic alterations of gene expression and failure in DNA repair, changes in receptor and metabolic pathways, and autophagy. However, the sources of ROS may differ in various cell types and may be dependent on cell-type functional and metabolic state.

1. **Nrf2 and redox balance during OA progression**

As is well known, aging is an important pathogenic factor in OA, and Nrf2 can play an indispensable role in inhibiting cellular aging through the antioxidant system. Research has shown that the activity of Nrf2 gradually decreases during the aging process of human fibroblasts. As expected, silencing Nrf2 is able to trigger premature aging, while pharmacologically activating Nrf2 can increase cell lifespan, indicating that in the context of oxidative stress, the inhibition of Nrf2 signaling is capable of facilitating premature cell aging [50]. Currently, numerous comprehensive studies have demonstrated that the role of Nrf2 transcription factor plays an essential role in maintaining cartilage homeostasis and regulating redox balance in OA. Antioxidant and detoxification enzymes, such as heme oxygenase 1 (HO-1), sulfiredoxin (Srx), thioredoxin reductase (TrxR), peroxiredoxins (Prxs), catalase, SODs, glutathione peroxidase (GPx), and NADPH:quinone oxidoreductase 1 (NQO1), can be significantly regulated and induced by Nrf2. Therefore, keeping the stability of Nrf2 physiological function exerts an essential effect in maintaining cellular redox homeostasis [51].Recent studies have validated that the activation of Nrf2 can decrease ROS production induced by IL-1β in chondrocytes, further suggesting that Nrf2 is a momentous determinant of antioxidant response [52, 53]. According to the study of *Y. Wang et al*, the level of Nrf2 protein in human OA chondrocytes has been decreased compared with healthy chondrocytes [54]. Interestingly, other researches have confirmed that Nrf2 protein levels are increased in OA cartilage and synovium compared with normal individuals [52, 53].Furthermore, *N.M. Khan et al* have proposed that the expression of Nrf2 gene is dramatically elevated in severely-injured OA cartilage compared to non-injured samples from the same OA joint [53].

Recently, it has been well acknowledged that Nrf2 plays an integral role in the maintain of cartilage homeostasis in vivo. Of note, *C.J. Wruck et al* have found and confirmed that in the mouse model of rheumatoid arthritis, Nrf2 knockout mice showed higher levels of oxidative stress and more severe articular cartilage damage, compared with wild-type mice [55]. Moreover, according to *D. Cai et al*, Nrf2 knockout mice exhibited more severe OA phenotype compared to wild-type mice in both post-traumatic OA models and inflammatory OA models [56]. Meanwhile, histone deacetylation inhibitors (TSA) have been shown to ameliorate the progression of OA in the above two mouse models via TSA-induced acetylation and Nrf2 activation pathways. In chondrocytes, downstream antioxidant mediators (such as HO-1) are activated by acetylation-induced Nrf2 and lead to up-regulation of its expression, which is related to the reduction of matrix metalloproteinase expression [56].HO-1 is considered as a vital downstream target of Nrf2, and recent evidences have indicated that knockout of Bach1 that is one of a transcriptional suppressor factor of HO-1, can alleviate the severity of age-related OA and surgical-induced OA in mice [57].In vitro studies, it has suggested that articular chondrocytes of Bach1 knockout mouse display higher levels of SOD2 protein, which is a pivotal superoxide detoxifying antioxidant. And, the level of SOD2 has been found to decrease in Bach1 knockout cells after HO-1 gene expression was silenced, indicating that the expression of SOD2 is dependent on HO-1. Besides, *T. Takada et al* have demonstrated that TBHP-induced chondrocytes apoptosis was enhanced when HO-1 expression was silenced, further proving that HO-1 exhibits an imperative effect in the dynamic redox balance of cartilage (**Figure 1**) [57].

Based on accumulating evidence, different kinds of compounds with antioxidant properties, such as Licochalcone A, Pterostilbene, Resveratroland, Wogonin and 6-gingero etc., have been identified to play anti-inflammatory as well as cartilage protective roles in the joint of OA via activating the Nrf2 signaling pathways (**Table 1**). In light of these findings, it is reasonable to speculate that Nrf2 signaling pathways contribute to the maintenance of redox balance and modulation of chondrocyte homeostasis in the process of OA, but the exact signaling pathways responsible for these effects are currently not fully explored. According to the results of *N.M. Khan et al*, Nrf2 may modulate IL-1β-induced ROS production by stimulating ERK MAP kinase pathway, thus promoting the anti-apoptotic effect, which further elucidates that the regulation of Nrf2-controlled signaling pathways may exert an important effect in attenuating oxidation and apoptosis of human OA chondrocytes [53].Additionally, with the in-depth study of Nrf2, clinical medicines targeting OA at present have not been found yet, but a variety of new clinical drugs have been developed for the treatment of other diseases. For example, based on a randomized, double-blind, placebo-controlled phase 3 clinical trial (CARDINAL), the Nrf2 activator Bardoxolone methyl has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of chronic kidney disease (CKD) caused by Alport syndrome [58]. Another Nrf2 activator, Dimethyl fumarate, can induce upregulation of antioxidant genes expression, and is therefore also approved by the FDA and the European Medicines Agency as a first‐line therapy for adult patients with relapsing‐remitting MS (RMSS) [59]. However, it has been shown that the activation of Nrf2 plays a double-edged role in cancers. On the one hand, Nrf2 can prevent the progression of cancers caused by oxidative stress, on the other hand, the specific activation of Nrf2 in different cancers can promote the proliferation of cancer cells as well as induce chemoresistance and radioresistance of cancer cells [60]. Therefore, potential therapeutic strategies accurately targeting Nrf2-regulated signaling pathways may have clinical value in ameliorating the progression of OA and much still remains to be done.

1. **Overall conclusion and future perspectives**

Accumulating evidences have suggested that OA is not only a chronic injury disease which is limited to joints, but also a comprehensive and degenerative disease involving multiple systems. A growing number of evidences support that oxidative stress exhibits a dramatic effect in age-associated alterations of articular cartilage, which disrupt cartilage homeostasis and contribute to the development OA. Aging, inflammation, along with mechanical load are capable of inducing oxidative stress and promoting ROS production, damaging proteins and DNA, which results in mitochondrial dysfunction, cell signaling conduction disorder, as well as changes in epigenetic gene expression. Therefore, high levels of ROS signaling pathways and altered Nrf2 activity can facilitate chondrocyte apoptosis along with cartilage degradation, and induce chondrocyte hypertrophy and subchondral bone dysfunction. What’s more, decreased Nrf2 activity may be a result of a failure in its homeostatic post-translational regulation and/or altered epigenetic and transcriptional regulatory mechanisms. A large amount of experimental evidence shows that Nrf2 plays a pivotal and complex role in the bone integrity, and many functions still need to be further explored. Treatment methods that increase Nrf2 activity may counteract oxidative stress in OA, thereby restricting cartilage degradation and bone resorption with effect, while the restoration of Nrf2 dynamic balance may induce the normalization of bone resorption and remodeling. Thus, investigating the effect of chondrocytes and Nrf2 in OA would provide a promising research field in developing a potentially therapeutic strategies for OA treatment.

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**Figure 1.** Nrf2 modulating oxidative stress in OA. Different negative stimulations including inflammatory components, mechanical overload, metabolic disorders and aging impair the function of the electron transport chain, leading to mitochondrial dysfunction. And then the ROS system is activated, thus inducing oxidative stress, which affects cell signaling conduction disorder via MAP kinase pathway, ultimately contributing to chondrocyte apoptosis and cartilage matrix degradation. And Nrf2 is able to modulate antioxidant and detoxification enzymes, such as heme oxygenase 1 (HO-1), sulfiredoxin (Srx), thioredoxin reductase (TrxR), peroxiredoxins (Prxs), catalase, SODs, glutathione peroxidase (GPx), and NADPH:quinone oxidoreductase 1 (NQO1), which inhibits the ROS system and thus reduces oxidative stress, further maintaining chondrocyte redox homeostasis and preventing the progression of OA.