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Commentary

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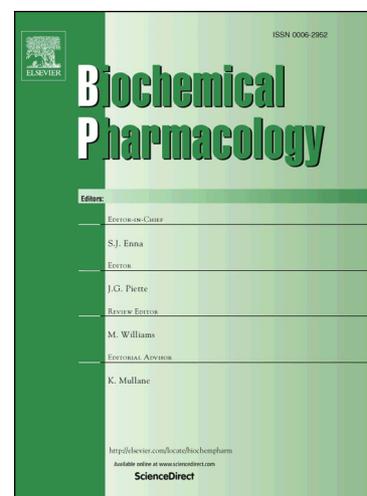
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Nrf2 as a therapeutic target for rheumatic diseases

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Abstract

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a master regulator of cellular protective processes. Rheumatic diseases are chronic conditions characterized by inflammation, pain, tissue damage and limitations in function. Main examples are rheumatoid arthritis, systemic lupus erythematosus, osteoarthritis and osteoporosis. Their high prevalence constitutes a major health problem with an important social and economic impact. A wide range of evidence indicates that Nrf2 may control different mechanisms involved in the physiopathology of rheumatic conditions. Therefore, the appropriate expression and balance of Nrf2 is necessary for regulation of oxidative stress, inflammation, immune responses, and cartilage and bone metabolism. Numerous studies have demonstrated that Nrf2 deficiency aggravates the disease in experimental models while Nrf2 activation results in immunoregulatory and anti-inflammatory effects. These reports reinforce the increasing interest in the pharmacologic regulation of Nrf2 and its potential applications. Nevertheless, a majority of Nrf2 inducers are electrophilic molecules which may present off-target effects. In recent years, novel strategies have been sought to modulate the Nrf2 pathway which has emerged as a therapeutic target in rheumatic conditions.

Keywords: Nrf2, rheumatic conditions, rheumatoid arthritis, systemic lupus erythematosus, osteoarthritis, osteoporosis

1. Introduction

The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway is involved in the regulation of many antioxidant, anti-inflammatory and cell survival genes. Activation of this signaling pathway which contributes to detoxification and protective processes has encouraged a wealth of studies on its potential health benefits and therapeutic applications. Nrf2 is a CNC-bZIP (Cap'n'collar-basic region leucine zipper) transcription factor [1]. In unstressed conditions, Nrf2 binds to the cytosolic inhibitor Kelch-like ECH-associated protein 1 (Keap1) an adaptor protein to the cullin-3 E3 ubiquitin ligase complex [2], which targets Nrf2 for proteasomal degradation. Nrf2 contains two Keap1 binding motifs, ETGE and DLG. Keap1 is a main regulator of Nrf2 and a sensor of oxidative and xenobiotic stress. Under basal intracellular redox conditions, Keap1 drives Nrf2 regulation but in the presence of cellular stress, Nrf2 can be regulated by both Keap1-dependent and -independent mechanisms [3]. Nrf2 accumulates in the nucleus and binds to the antioxidant-response element (ARE) sites in the promoter of target genes as a heterodimer with a small musculoaponeurotic fibrosarcoma (sMaf) protein (Figure 1). In addition, Nrf2 has been reported to form heterodimers with proteins such as c-Jun, activating transcription factor 4, and others depending on cell type and stimuli [4,5]. Genes targeted by Nrf2 include genes involved in the synthesis and conjugation of glutathione, heme and iron metabolism, drug metabolism and transport, as well as antioxidant proteins, enzymes and transcription factors. Nrf2 is essential for the transcriptional induction of phase II detoxifying enzymes and antioxidant proteins which represent a main defense mechanism. For instance, glutathione reductase, glutathione S-transferase, γ -glutamylcysteine synthetase,

NAD(P)H:quinone oxidoreductase-1 (NQO1) and heme oxygenase-1 (HO-1) (reviewed in [6]).

Oxidants and electrophiles modify Keap1 cysteine residues which causes a conformational change in this protein leading to cessation of Nrf2 polyubiquitination. Then, Nrf2 translocates to the nucleus to initiate the transcription of target genes [7,8]. Phosphorylation mediated by glycogen synthase kinase 3 (GSK-3) creates a recognition motif for the E3 ligase adapter β -transducin repeat containing E3 ubiquitin protein ligase (β -TrCP) leading to an alternative pathway for ubiquitin-dependent proteasomal degradation of Nrf2 [3].

Nrf2 can be regulated by acetylation by p300/cAMP response element-binding protein (CREB)-binding protein (CBP) [9]. Thus, acetylation of Nrf2 results in binding to the ARE and activation of gene transcription, whereas deacetylation releases it leading to transcriptional termination and nuclear export [10]. Nrf2 acetylation is determined by the relative activities of histone acetyl transferases and histone deacetylases (HDACs) [9]. Bach proteins dimerize with sMaf proteins and these complexes compete with Nrf2-sMaf. In particular, Bach1 plays significant roles by activating and repressing transcriptional activities to regulate the oxidative stress response and suppress HO-1 [11].

Rheumatic diseases are chronic conditions affecting the musculoskeletal system. Arthritis and related illnesses cause inflammation, changes in the joints, pain and limitations in motion and function. These conditions have a profound effect on work capacity and quality of life of affected people. In the industrialized world rheumatic diseases affect more individuals than any other disease group (European League Against Rheumatism: www.eular.org, and American College

of Rheumatology: www.acr.org) and constitute a major health problem with an important social and economic impact. The burden related to these conditions is expected to increase in the near future as the population ages.

In recent years, several lines of evidence have supported the notion that Nrf2 plays a regulatory role not only in oxidative stress, but also in inflammation, immunity and cartilage and bone metabolism. The results of many *in vitro* and *in vivo* studies have led to propose that Nrf2 activation may control different processes and mediators involved in the pathophysiology of rheumatic conditions. Some relevant examples can be rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), osteoarthritis (OA) and osteoporosis. The aim of this Commentary is to focus on Nrf2 as a new therapeutic target in these conditions.

2. Nrf2 regulation of inflammatory and immune responses

A wide range of evidence indicates that Nrf2 plays an important role in the regulation of inflammation as well as in innate and adaptive immune responses. The control of redox activity by Nrf2 and antioxidant downstream targets may play a role in the activation of the NLR family, pyrin domain containing 3 (NLRP3) inflammasome. NLRP3 is involved in homeostasis and tissue repair although its dysregulation contributes to inflammatory and degenerative diseases [12]. The activation of the cytosolic inflammasome complex results in inflammatory caspase activation leading to the secretion of interleukin(IL)-1 β and IL-18 mainly in monocytes/macrophages and dendritic

cells (DCs) [13]. Increased NLRP3 inflammasome activity is a key feature of both autoinflammatory and autoimmune diseases [14] and Nrf2 has recently been proposed as a potential target for the therapeutic modulation of NLRP3-associated diseases [15].

Nevertheless, Nrf2 deficiency in mice results in defective activation of NLRP3 and absent in melanoma 2 (AIM2) inflammasomes in bone marrow macrophages after treatment with a variety of stimuli including monosodium urate and non crystalline agents [16]. In contrast, it has been reported that Nrf2 is an inhibitor of NLRP3 expression at the transcriptional level and thus, Nrf2 activation inhibited lipopolysaccharide-induced NLRP3 production in THP1 cells [15]. These conflicting findings clearly emphasize the need of studying the precise molecular mechanisms involved in Nrf2 interaction with inflammasomes.

Nrf2 not only inhibits inflammation through redox control but also downregulates pro-inflammatory cytokines, chemokines, adhesion molecules and enzymes. Nrf2 and HO-1 have shown the ability to control the migration of inflammatory cells, a key process in the development of chronic inflammatory conditions. The inhibition of adhesion molecules and matrix metalloproteinase (MMP) expression can mediate these anti-inflammatory effects [17,18].

Nrf2 activity is essential to control cellular mechanisms contributing to the resolution of the inflammatory process. For this purpose, Nrf2 interplays with nuclear factor- κ B (NF- κ B) through multiple molecular interactions (reviewed in [19]). Phosphorylation of NF- κ B inhibitor (I κ B α) by I κ B kinase (IKK β) leads to I κ B α degradation which results in nuclear translocation and DNA binding of NF- κ B. Hydroperoxides can regulate NF- κ B activation by several mechanisms. For instance, in basal redox conditions, Keap1 is responsible for IKK β ubiquitination

and degradation but in the presence of oxidants, Keap1 is inhibited and NF- κ B can be activated [20]. Nrf2 decreases NF- κ B activation by interacting with Keap1. In addition, Nrf2 can interact with the sMaf protein MafK. This protein enhances the acetylation of p65 and thus the DNA-binding activity of NF- κ B. Therefore, Nrf2 may maintain low levels of MafK avoiding excessive p65 acetylation [21].

Nrf2 contains several κ B sites in its proximal promoter, which are subjected to regulation by p50 and p65. In turn, NF- κ B may play a dual role in the regulation of Nrf2 activity. NF- κ B activation induces Nrf2 expression in cells such as human monocytes and acute myeloid leukemia cells, leading to enhanced activation of Nrf2-dependent antioxidant defense responses but it can also inhibit Nrf2 by several mechanisms such as the competition for the transcriptional co-activator p300/CBP. In addition, NF- κ B increases the recruitment of HDAC3 to the ARE region and thus Nrf2 transcriptional activation is prevented [18].

Nrf2 activation promotes the resolution of inflammation through the induction of prostaglandin (PG) D synthase expression in macrophages leading to the rapid production of PGD₂/15-deoxy-delta(12,14)-prostaglandin J₂ (15d-PGJ₂) which sustains a positive feedback loop to limit the inflammatory response. It has been shown that 15d-PGJ₂ activates Nrf2 leading to the induction of CD36 and HO-1 in macrophages to promote efferocytosis and the resolution of inflammation [22].

Nrf2 deficiency induces autoimmune phenotypes in certain strains of mice and increases susceptibility to the development of autoimmune diseases [23] whereas Nrf2 activation is associated with the attenuation of these

conditions [17,24]. In the presence of Nrf2 dysregulation, oxidative tissue damage and apoptosis could increase the production of autoantigens leading to activation of T cells and production of autoantibodies by B cells. In addition, loss of phase II enzymes causes an elevation in the steady state of reactive intermediates by failing to remove them. This can promote the activation of immune cells. As Nrf2 is a master regulator of cellular responses against environmental stresses [8], it is likely that Nrf2 activation can protect against environmental factors contributing to autoimmune pathogenesis.

Nrf2-mediated regulation of autoimmune function can involve the suppression of pro-inflammatory T helper(Th)1 and Th17 cell responses, and the enhancement of anti-inflammatory Th2, regulatory T cells (Treg) and regulatory B cells functions (Figure 2). In addition, Nrf2 may control the differentiation and function of DCs and macrophages. Nrf2 deficiency alters the function and phenotype of DCs with increased co-stimulatory molecule expression and enhanced antigen-specific T cell stimulatory capacity in immature cells [25].

The influence of Nrf2 on T cell function is complex. Disruption of Nrf2 limits glutathione availability leading to the inhibition of antigen-induced CD8+ T cell proliferation and function [26]. In contrast, Nrf2 activation inhibits secretion of the Th1 cytokines, interferon γ (IFN- γ) and tumor necrosis factor α (TNF α), promotes early production of IL-2 [27] and skews CD4+ T cells toward Th2 differentiation [28]. Recently, clustered regularly interspaced short palindromic repeats (CRISPR) technology has been used for *ex vivo* Keap1 editing in primary human T cells in order to achieve Nrf2 activation and enhanced anti-inflammatory and immunosuppressive functions [29].

The inhibition of transcription of inflammatory mediators may contribute to the therapeutic effects of Nrf2 activation in autoimmune diseases. It also appears that HO-1 induction may contribute to the immunosuppressive ability of Nrf2 activation. It is known that HO-1 and CO reduce the capacity of antigen-presenting cells, such as DCs and macrophages to recognize pathogen-associated molecular patterns thus suppressing both antigen presentation and the production of pro-inflammatory cytokines [19]. HO-1 activity was also found to modulate the proliferative capacity of T cells, the effector functions of T cells and natural killer cells, and the suppressive functions of Treg [30].

The immunoregulatory effects of Nrf2 have been classically related to the control of oxidative stress and phase II enzymes, glutathione levels or NF- κ B activation [19]. Nevertheless, some data suggest the contribution of other mechanisms such as the p38-CREB/activating transcription factor 1 (ATF1) signaling axis in DCs [25] or the disruption of RNA polymerase II recruitment which results in the inhibition of IL-6 and IL-1 β transcription in macrophages [17].

3. Rheumatoid arthritis

RA is a chronic autoimmune disease characterized by synovial hyperplasia, immune cell infiltration and degradation of cartilage and bone. There are activation and migration of neutrophils, macrophages, and lymphocytes which result in the increased production of pro-inflammatory mediators such as oxidants, eicosanoids, cytokines (IL-17, TNF α , IFN- γ , IL-6, and IL-1 β) and catabolic enzymes, with hyperproliferation of synovial fibroblasts

[31]. This results in joint swelling and progressive destruction of cartilage and bone. Excessive oxidant generation may contribute to the pathogenesis of RA. In fact, RA patients show a marked increase in lipid peroxidation, protein oxidation and DNA damage associated to a reduced activity of the antioxidant defense system, which may contribute to tissue damage and the perpetuation of disease [32]. As a response to oxidative stress, Nrf2 expression is activated in synovial cells from RA patients and also in joints of antibody-induced arthritic mice although this response is not enough to counteract arthritis progression [33]. In fact, enhanced gene expression of Nrf2/HO-1 in a subgroup of RA patients has been related to more severe disease state with an underlying lack of apoptosis in synovial fibroblasts, macrophages, lymphocytes, and other cells that may contribute to the persistence of RA [34].

Nrf2 deficiency enhances joint alterations in experimental RA models. In K/BxN serum transfer arthritis and antibody-induced arthritis, Nrf2 deletion accelerates the incidence and aggravates the disease, with important inflammation and lesions [33,35]. Nrf2 deficiency dramatically upregulates oxidative stress, cell migration, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase expression, the pro-inflammatory cytokines TNF α and IL-6, as well as the chemokine CXCL-1. In addition, we showed that Nrf2 may be a protective factor for bone metabolism in the presence of arthritis [35]. On the contrary, Nrf2 activation and HO-1 induction exert anti-inflammatory and antioxidant effects in animal models of RA and in human RA synovial fibroblasts [36,37].

Many molecules have shown anti-inflammatory and immunoregulatory properties via the oxidative stress-responsive transcription factor Nrf2. There is

a variety of Nrf2 inducers, most of which are electrophilic and react with cysteine thiols of Keap1. In particular, Cys151, Cys273, and Cys288 play an important role in Nrf2 activation. Therefore, modification of thiol moieties in Keap1 leads to disturbance of the structure and the decline of ubiquitin ligase activity [38]. As a main example, sulforaphane (SFN, Figure 3) can exert immunoregulatory effects leading to the inhibition of T cell proliferation and the production of IL-17 and TNF α by RA CD4⁺ T cells [39]. This compound is also able to polarize pro-inflammatory M1 macrophages into anti-inflammatory M2 cells [40]. In cultured human synoviocytes, SFN induced apoptosis by modulating the expression of Bcl-2/Bax, p53, and pAkt and inhibited inflammation [39]. Intraperitoneal administration of SFN to mice reduced the clinical severity of collagen-induced arthritis, anti-collagen II antibody levels, T cell responses and the production of IL-17, TNF α , IL-6, and IFN- γ by lymph node cells and spleen cells [39]. Part of the anti-inflammatory effects of SFN would be dependent on Nrf2 activation which indirectly inhibits NF- κ B via HO-1-mediated CO production but part of them are independent of Nrf2 as SFN can directly inhibit NF- κ B [41].

The Nrf2 activator and immunoregulatory agent dimethyl fumarate (DMF) is used in systemic sclerosis and severe plaque psoriasis. It has been reported that DMF partly exerts its anti-inflammatory effects via inflammasome inhibition [42]. Furthermore, blockade of T cell activation by DMF may be related to binding specific cysteine residues in protein kinase C (PKC) θ which is a key kinase in T cell signaling [43], and DMF and monomethylfumarate (MMF) may activate the hydroxycarboxylic acid receptor 2 (HCAR2) which results in downregulation of NF- κ B [44].

Many studies have reported the effects of polyphenols, and mainly of the green tea's active ingredient, epigallocatechin 3-gallate in preclinical models of RA as well as *in vitro*, in cartilage and bone protection and synovial fibroblast regulation. This compound induces Nrf2 and HO-1 in different cell types while decreasing NF- κ B activity and the production of inflammatory and extracellular matrix degradative mediators [45]. The activation of synovial fibroblasts and the production of inflammatory cytokines are crucially involved in the pathogenesis of RA. It is interesting to note that calycosin, an isoflavone from the Chinese medicinal herb Radix Astragali, has been reported to downregulate pro-inflammatory cytokines and COX-2 via p62-Nrf2-HO-1 induction in RA synovial fibroblasts [46].

Antirheumatic gold(I)-containing compounds stimulate the antioxidative stress response through activation of Nrf2/sMaf leading to the upregulation of HO-1 and γ -glutamylcysteine synthetase [47]. Nrf2-HO-1 activation also mediates the anti-inflammatory effects of H₂S and related compounds which are able to modify by sulfydrylation the cysteine residue of Keap1. For instance, the endogenous H₂S modulator S-propargyl-cysteine is able to reduce the generation of inflammatory mediators, oxidants, and MMP-9 as well as the cell invasive activity in rheumatoid fibroblast-like synoviocytes MH7A. *In vivo*, this compound ameliorated the severity of arthritis in the adjuvant model in rats [37].

Oxidized phospholipids able to regulate antioxidant gene expression via Nrf2 signaling *in vivo* have been detected under chronic inflammatory conditions. Interestingly, epoxycyclopentenone derivatives have been shown to activate Nrf2 leading to inhibition of pro-inflammatory cytokines and chemokines in myeloid cells. These effects were similar to those of the pro-resolving lipid

mediator 15d-PGJ₂ which has been reported to interact with the nuclear hormone receptor peroxisome proliferator-activated receptor- γ (PPAR- γ) as well as with Nrf2. Of note, 15d-PGJ₂ was active in PPAR- γ -deficient cells but not in Nrf2-deficient cells implying that the anti-inflammatory activity of 15d-PGJ₂ was mediated via Nrf2 rather than PPAR- γ [48]. 15d-PGJ₂ forms an adduct to Keap1 and disrupts Nrf2 ubiquitination, leading to the accumulation of Nrf2 in the nucleus. In addition, 15d-PGJ₂ ameliorated adjuvant-induced arthritis with suppression of pannus formation and mononuclear cell infiltration [49].

Polyunsaturated fatty acids are able to inhibit inflammation in macrophages through the induction of Nrf2 [50]. It is interesting to note that eicosapentaenoic acid and docosahexaenoic acid inhibit inflammatory cartilage degradation. The last compound also ameliorates disease activity in patients with RA and enhances plasma levels of pro-resolving maresin/resolvin precursors [51].

4. Systemic lupus erythematosus

An increased production of oxidative stress may contribute to immune cell death and autoimmunity in SLE. Necrosis secondary to deregulated cell death and removal processes results in the generation of autoantigens and formation of immune complexes which induce inflammation and tissue damage in organs such as the kidney, skin and joints. SLE patients show alterations in repair mechanisms of oxidative DNA damage, high serum levels of oxidized proteins, apoCIII, oxidized phospholipids and autoantibodies to oxidatively modified lipoproteins [52].

Nrf2 polymorphisms have not been associated to lupus susceptibility although the Nrf2 -653 G/A polymorphism is related to the risk of nephritis among Mexican childhood-onset female SLE patients [53]. Increased levels of Nrf2, NQO1 and 8-oxo-7,8-dihydro-2'-deoxyguanosine were observed in glomeruli from human lupus nephritis. Nrf2 is also induced in other types of nephritis and may result from immune-complex deposition [54].

Female mice deficient in Nrf2 develop with age a multi-organ autoimmune disorder similar to SLE with increased DNA oxidation and lipid peroxidation, splenocyte apoptosis, presence of antibodies against dsDNA and the Smith antigen, and tissue damage (vasculitis, glomerulonephritis, hepatitis, and myocarditis) [55]. According to the genetic background of mice, there are differences in the age necessary to develop these changes [23]. Although oxidative damage due to Nrf2 deficiency is present in both male and female mice, only female mice show progression to SLE suggesting that gender-specific factors are involved in breaking of immune tolerance to self antigens [55]. In addition to deficiency in phase 2 detoxification enzymes and antioxidant genes in hepatic and lymphoid cells which results in oxidative damage to tissues, Nrf2 knock-out results in enhanced proliferative responses of CD4+ T cells, altered CD4+/CD8+ ratios and promotion of Th17 cells differentiation and function [56]. Nrf2 deletion has also been shown to promote Th17 differentiation and function during lupus nephritis development by regulating the suppressor of cytokine signaling 3 (SOCS3)/signal transducer and activator of transcription (STAT) 3 pathway and IL-1 β [56].

Nrf2 inducers inhibit the development of disease in animal models such as pristane-induced lupus nephritis and spontaneous lupus in MRL/lpr mice.

Therefore, induction of Nrf2 by SFN protects renal cells from developing lupus nephritis by downregulation of oxidative stress and inhibition of NF- κ B and extracellular matrix deposition [57]. Interestingly, fumaric acid esters have been used as systemic combination therapy in the treatment of severe, extensive and recalcitrant cutaneous manifestation of SLE. They were well-tolerated and showed excellent efficacy and a steroid-sparing effect [58], supporting the interest of this approach in the therapy of lupus. As another example, epigallocatechin-3-gallate prevents lupus nephritis development via the upregulation of the Nrf2 antioxidant pathway, which inhibits NLRP3 inflammasome activation [59].

5. Osteoarthritis

OA is characterized by a progressive cartilage degradation associated with hypertrophic differentiation of chondrocytes, synovitis and alterations in subchondral bone and periarticular tissues. Long-time exposure to a low-grade chronic inflammation concomitant with a failure in oxidant-antioxidant balance has an important impact on the pathogenesis of disease. Catabolic and pro-inflammatory mediators are produced by the inflamed synovium leading to excess production of proteolytic enzymes responsible for cartilage breakdown [60].

Mitochondrial dysfunction and oxidative damage are involved in the pathogenesis of OA. Oxidative stress is involved in the production of inflammatory and catabolic mediators and also contributes to joint degradation

by mechanisms such as the reduction in extracellular matrix synthesis, the induction of chondrocyte apoptosis and MMP activation [61]. Therefore, the control of oxidative stress and chronic inflammation by Nrf2 would result in protective effects against joint alterations in OA (Figure 4). Furthermore, as revised in the osteoporosis section, Nrf2 is an important factor to regulate the balance between osteoclast-driven bone resorption and osteoblast-driven remodeling which may play a role in the control of bone metabolism in OA.

The appropriate expression and balance of Nrf2 is necessary for normal chondrogenesis and regulation of cartilage metabolism. In fact, sustained overexpression of Nrf2 can inhibit chondral differentiation markers as collagen II, collagen X and osteopontin [62], but downregulation of Nrf2 could result in inhibition of chondrogenesis through apoptotic cell death [63]. In agreement with these findings, Nrf2 and glutathione transferase A4-4 expression is significantly lower in OA cartilage from humans and mice in comparison to normal controls [64].

The control of excessive oxidative stress and pro-inflammatory and catabolic mediators may sustain a protective role of Nrf2 in OA. In this context, Nrf2 knock-out mice display more severe cartilage damage in both the monoiodoacetate (MIA) and the surgical destabilization of medial meniscus (DMM) models of OA [65]. Induction of HO-1 by Nrf2 activation can also play a role in its anti-inflammatory and chondroprotective effects as HO-1 is able to reduce NF- κ B activity and inflammatory and degradative mediators in OA chondrocytes, synoviocytes and osteoblasts [66-68].

Recent investigations have shown that induction of Nrf2 by protandim (a commercial dietary supplement composed of five antioxidant phytochemicals)

and 6-gingerol mediates their protective effects in OA chondrocytes, where both drugs were able to attenuate the production of oxidative stress and inflammatory mediators as well as 6-hydroxynonenal-induced cell death. In addition, protandim administration to mice significantly reduced joint destruction in the DMM [64]. Piceatannol is another Nrf2 inducer with protective effects in this model of OA that also inhibits the production of inflammatory mediators, MMP-13 and aggrecanase-2 in OA chondrocytes stimulated with IL-1 β [69]. Recently, the natural flavonoid wogonin has been reported to exert anti-inflammatory and protective effects in human OA chondrocytes and cartilage explants [70]). Wogonin modulates the oxidant-mediated activation of Nrf2 signaling axis and also disrupts Keap 1/Nrf2 interaction by blocking the binding site of Nrf2 in Keap 1 protein.

Nrf2 acetylation, mediated by histone acetyltransferase/HDAC enhances its transcriptional ability and the expression of downstream targets. Therefore, inhibition of HDAC results in Nrf2 activation. This is the mechanism of action of trichostatin A, a pan-HDAC inhibitor which protects against cartilage degradation and inflammation in the MIA and DMM models of OA through the induction of Nrf2 in joint tissues [65]. Besides trichostatin A, other HDAC inhibitors such as sodium butyrate and vorinostat have been shown to reduce inflammatory responses and the upregulation of MMPs and aggrecanase 2 in human OA chondrocytes. As a result, HDAC inhibitors have demonstrated protective effects against cartilage degradation through mechanisms such as Nrf2 activation and the inhibition of NF- κ B and MAPK [71].

Thus, strategies aimed at stimulating antioxidant gene expression, as HO-1 and NQO-1, through Nrf2 activation in aging cartilage may hold promise

for OA therapy [72]. Nevertheless, it should be taken into account that some Nrf2 inducers can protect against cartilage degradation by other mechanisms. For instance, SFN inhibited cytokine-induced MMP expression in human articular chondrocytes and fibroblast-like synovial cells independently of Nrf2 and HDAC activity [73].

6. Osteoporosis

Formation and maintenance of bone tissues are regulated by two main mechanisms, bone formation by osteoblasts and bone resorption by osteoclasts. Many factors can disturb bone remodeling and break the balance between bone resorption and formation, which contribute to osteoporosis. Activation of immune cells in chronic inflammation results in an excessive production of bone-resorbing cytokines which are major stimulators of osteoclastogenesis. Elevated oxidative stress contributes to alterations in bone metabolism. As a consequence, there is systemic or local bone loss associated with osteoporosis, RA, etc. [74].

Nrf2 is one of the transcription factors responsible for the regulation of differentiation and function of osteoblasts and osteoclasts in normal bone metabolism. The results from different studies indicate that Nrf2 is indispensable for normal bone microarchitecture and suggest a role for this transcription factor in the maintenance of bone integrity in pathological situations.

Nrf2 plays an essential role in bone regeneration as Nrf2 knock-out mice show impaired fracture healing [75]. The Keap1/Nrf2 axis regulates receptor activator of nuclear factor κ -B ligand (RANKL)-dependent osteoclastogenesis via expression of enzymes such as HO-1, γ -glutamylcysteine synthetase, and glucose-6-phosphate dehydrogenase which modulate intracellular oxidative stress signaling [76]. Nrf2 deficiency promotes osteoclast differentiation mediated by increased oxidants production and activation of MAPKs and nuclear factor of activated T-cells 1 leading to bone resorption [77]. These animals show enhanced RANKL and osteoclast numbers accompanied by a decrease in osteoblast mineralization which increase their susceptibility to radiation-induced bone loss [78].

However, the effects on osteoblast metabolism are more complex. A high level of oxidative stress exerts negative effects on osteoblast metabolism and results in cell damage which suggests a protective role for Nrf2. Nevertheless, there are conflicting reports as Nrf2 regulates the antioxidant endogenous response and bone accrual differently depending on factors such as sex and age [79]. Stable overexpression of Nrf2 exerts negative effects on MC3T3 osteoblastic cells differentiation through inhibition of Runx2-dependent transcriptional activity [80]. In experiments using Nrf2 knock-out mice, it was concluded that Nrf2 exerts inhibitory effects on osteoclastic and osteoblastic differentiation with a higher effect on osteoblasts, in 9-week old mice [81]. In contrast, it has been reported that female Nrf2 knock-out mice exhibit a marked deficit in postnatal bone acquisition, by 3 weeks, related to a low osteoblast number and increased oxidants production which might impair early osteoblastogenesis and lead to the failure of bone acquisition [82]. In line with

these results, we have shown that Nrf2 deficiency in female mice leads to increased oxidative stress, bone turnover and bone resorption as a result of the predominance of osteoclastic activity over osteoblastic activity [83].

In addition, data from a model of osteoporosis in ovariectomized mice suggest a role for Nrf2 in bone anabolic effects, as an increase in HDAC2 by miR-455-3p inhibited the activation of Nrf2/ARE leading to increased oxidative stress and inhibition of osteoblast growth exacerbating osteoporosis [84].

Nrf2 inducers may be useful as inhibitors of bone destruction. As an example, DMF is able to inhibit RANKL-mediated osteoclastogenesis and attenuate bone destruction in lipopolysaccharide-treated mice [85]. SFN, epigallocatechin gallate [86], carnosic acid [87], caffeic acid phenethyl ester [88] and an ETGE-peptide [89] can also inhibit osteoclastogenesis through Nrf2 activation while mangiferin protects osteoblasts from oxidative stress by this mechanism [90].

Activation of Nrf2 has been suggested as a therapeutic target to avoid glucocorticoid-induced osteoporosis. Thus, indole-3-carbinol, a natural product found in broadly consumed plants of the *Brassica* genus [91], alpinumisoflavone [92] and icariside II [93] block oxidants overproduction and osteoblast apoptosis induced by dexamethasone through Nrf2 induction.

Another strategy to control excessive bone resorption may be the induction of Bach1 nuclear export which activates Nrf2-dependent antioxidant enzyme expression leading to the attenuation of osteoclastogenesis [94].

7. Perspectives

Our understanding of the significance of Nrf2 activation for the control of human disease has so far been greatly hampered by the complexity of signaling pathways and biological responses modified by this transcription target as exemplified by the knowledge of Nrf2 interactome, regulome and fine-tuned regulatory loop [95]. As a consequence, there are discrepancies with regard to the reported biological effects of Nrf2 modulation although most results primarily reveal an anti-inflammatory effect.

At present, the possible crosslink between the Nrf2-ARE pathway and NLRP3 inflammasome is not well understood. Further in-depth biochemical analysis about Nrf2 and inflammasome interactions should provide the mechanistic insights necessary to establish the potential interest of Nrf2 in inflammasome-related diseases.

The regulation of Nrf2 opens up new therapeutic opportunities for the treatment of rheumatic conditions. Classic Nrf2 activators mimic the endogenous process of Nrf2 activation by covalent modification of cysteine groups in Keap1. At the same time, they can exert non-specific effects through the covalent modification of nucleophilic groups in proteins. Many types of post-translational oxidation reactions are thus possible leading to protein changes in conformation and activity in a wide range of kinases, phosphatases, transcription factors, transporters and cytoskeletal proteins [96]. As a consequence, significant side effects can be caused by these drugs. In this regard, less electrophilic derivatives such as MMF and monoethyl fumarate may be safer drugs than DMF [97]. Therefore, chemical modification of classic Nrf2

activators can lead to the development of potential therapeutic agents based on the Nrf2 pathway.

Another crucial aspect is the need for efficient control of intensity and duration of Nrf2 activation as it has been demonstrated that high-level and/or long-term activation of Nrf2 and its target genes, which are associated with the stress response in a normal physiological context, may result in deleterious effects such as growth of cancer cells and chemoresistance [38]. These observations support the importance of approaches for inducible/transient expression of cytoprotective enzymes which can be achieved with some drugs such as synthetic triterpenoids [98].

Other strategies focus on non-canonical mechanisms of Nrf2 activation. Nrf2 interaction with Keap1 can be disrupted by protein-protein interaction inhibitors such as peptide antagonists. Therefore, the peptide DEETGE-CAL-Tat has been shown to effectively activate Nrf2 and protect against cerebral ischemia in a pre-clinical model [99]. In addition, small-molecule Keap1–Nrf2 protein-protein interaction inhibitors have been designed [100].

Activation of Nrf2 can be achieved by Bach1 gene knockout which has shown immunoregulatory and protective effects in different disease models suggesting that drugs binding Bach1 may be a novel strategy to enhance Nrf2 activity [96] which may be useful in the control of autoimmune diseases.

There is a need of selective Nrf2 activation in target cells thus avoiding indiscriminated activation throughout the body and possible toxicity. In this respect, a number of pro-drugs able to be converted into Nrf2-activating molecules in the presence of oxidative stress or specific enzymes are under study [96]. In addition, the application of new technologies such as CRISPR

have the potential for clinical translation and can help to understand the mechanisms involved in Nrf2 effects in rheumatoid conditions.

These attractive strategies related to the Nrf2 pathway need to be validated to allow the development of new therapeutic agents. Activation of Nrf2 may also lead to adjuvant drugs thus helping improve cellular responses to other treatments. More studies are needed for a deep understanding of Nrf2 mechanisms and effects as a necessary step before entering clinical trials of rheumatic diseases.

On the other hand, the development of biomarkers provides objective parameters for diagnostic or prognostic purposes. Clinically validated biomarkers are needed in rheumatic diseases largely in early phases of disease. Nrf2 activation and expression of its target genes have been considered as biomarkers of oxidative stress in different pathological states such as cancer or multiple sclerosis. However, the potential of this pathway to become a biomarker for rheumatic diseases is not known and it should be explored.

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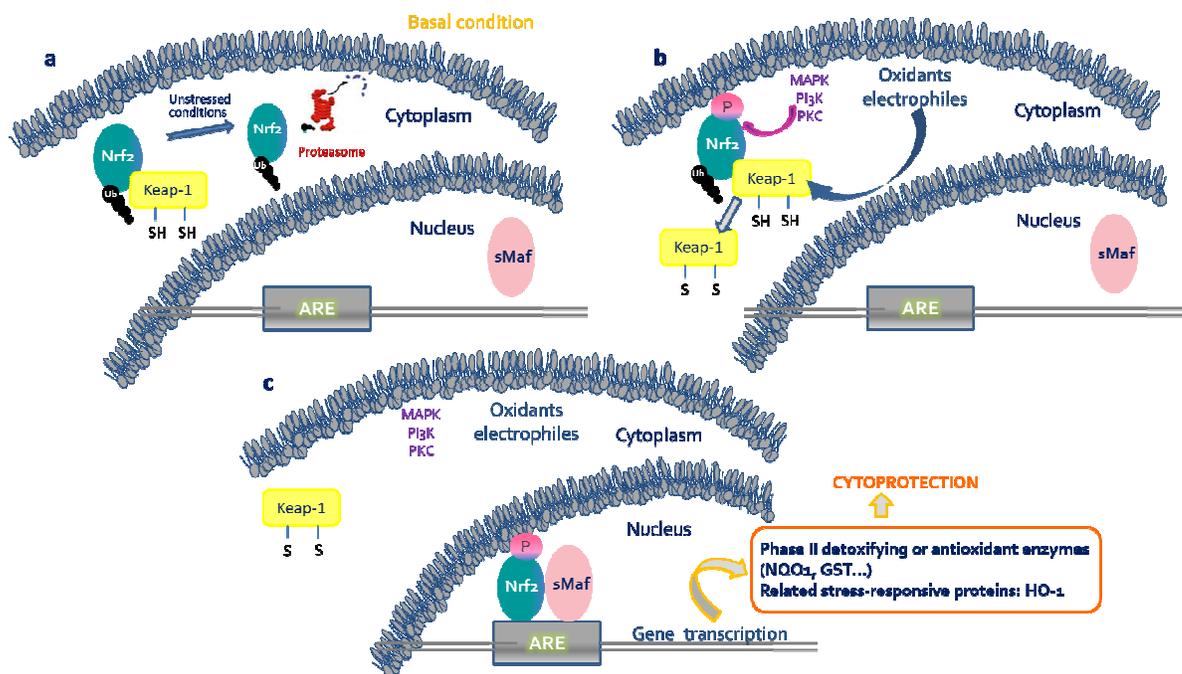
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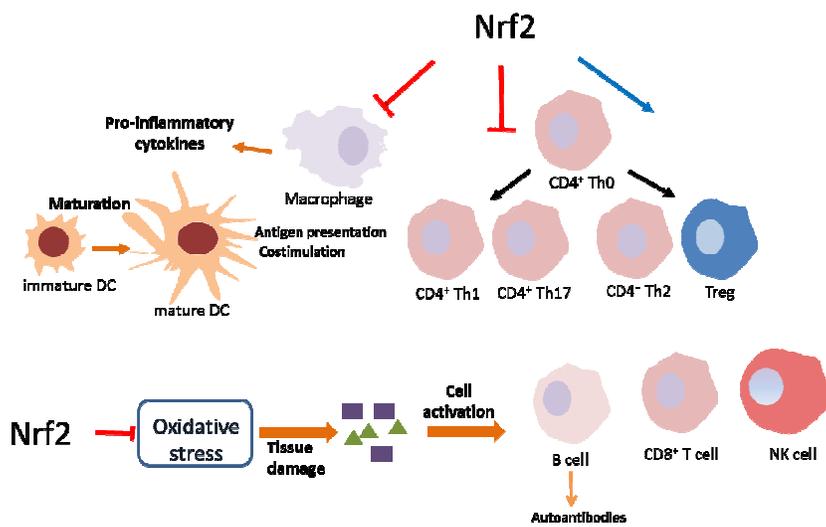
Figure 1. Nrf2 activation: a) basal conditions; b) activation by electrophiles or oxidative stress; c) nuclear translocation and gene transcription. ARE, antioxidant response element; GST, glutathione-S-transferase; HO-1, heme oxygenase-1; Keap-1, Kelch-like ECH-associated protein 1; MAPK, mitogen-activated protein kinase; NQO1, NAD(P)H:quinone oxidoreductase-1; PI3K phosphoinositide 3-kinase; PKC, protein kinase C; sMaf, small musculoaponeurotic fibrosarcoma; Ub, ubiquitin.

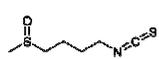
Figure 2. Regulation of the immune response by Nrf2. In blue: enhancement; in red: inhibition (DC, dendritic cell; NK, natural killer cell; Th, T helper cell; Treg, T regulatory cell).

Figure 3. Structures of representative Nrf2 inducers.

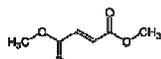
Figure 4. Nrf2 effects in joint cells. In blue: enhancement; in red: inhibition. MMPs, matrix metalloproteinases; NO, nitric oxide; PGs, prostaglandins; PMN, polymorphonuclear leukocytes; RANKL, receptor activator of nuclear factor- κ B ligand.



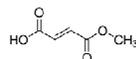




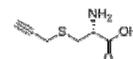
Sulforaphane



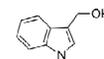
Dimethyl fumarate



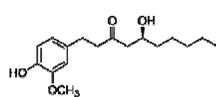
Monomethyl fumarate



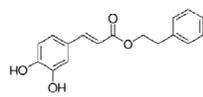
S-propargyl-cysteine



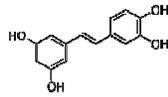
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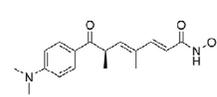
6-Gingerol



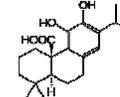
Caffeic acid phenethyl ester



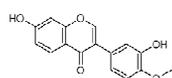
Piceatannol



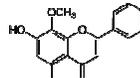
Trichostatin A



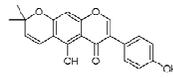
Camosic acid



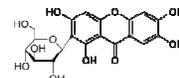
Calycosin



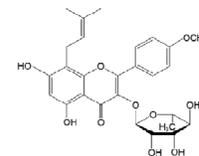
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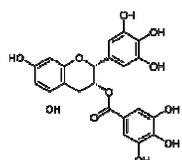
Alpinumisflavone



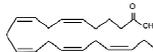
Mangiferin



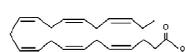
Icariside II



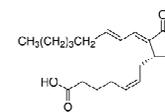
Epigallocatechin 3-gallate



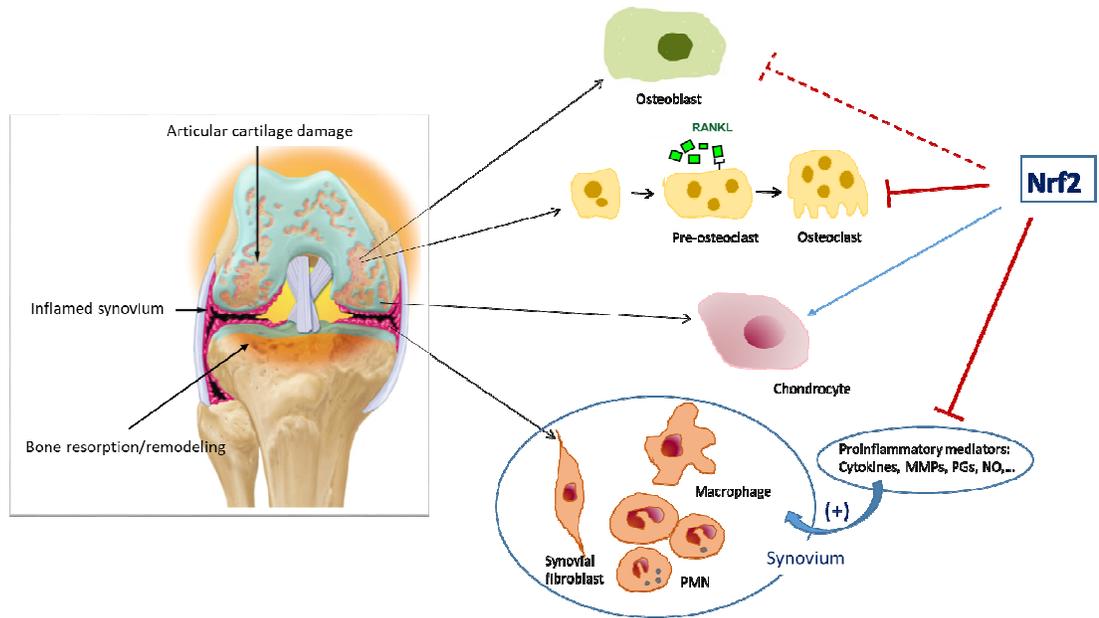
Eicosapentaenoic acid



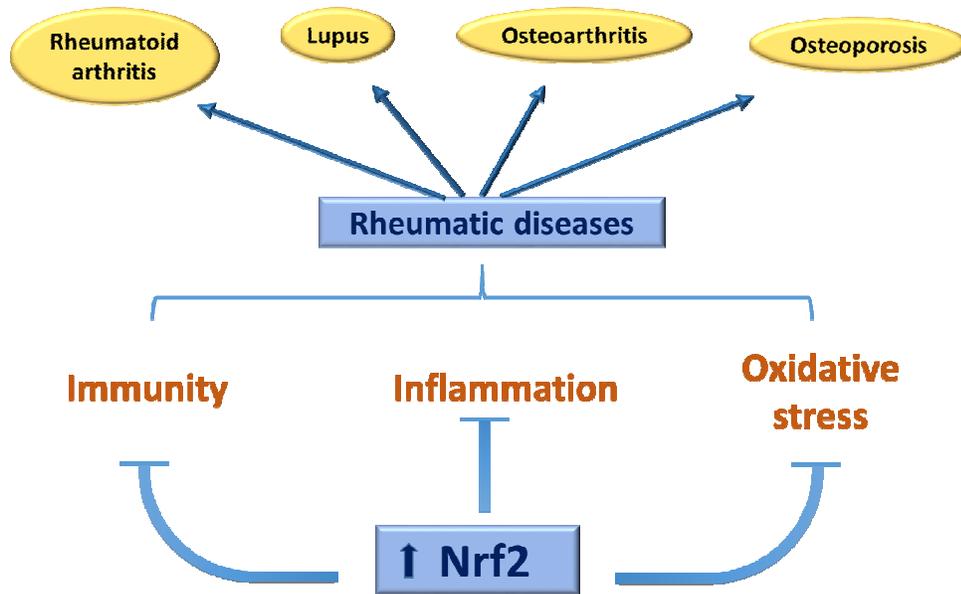
Docosahexaenoic acid

15d-PG₂

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