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Review

An Overview of Therapeutic Targeting of Nrf2 Signaling Pathway in Rheumatoid Arthritis

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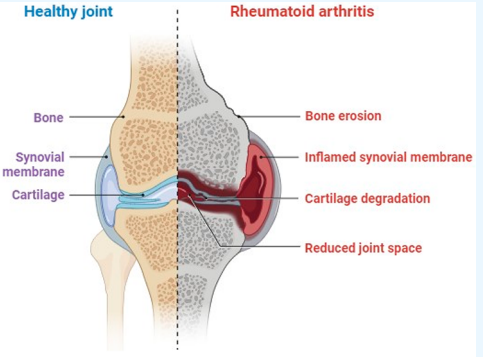
ABSTRACT: Rheumatoid arthritis (RA), an autoimmune condition that has a significant inflammatory component and is exacerbated by dysregulated redox-dependent signaling pathways. In RA, the corelationship between oxidative stress and inflammation appears to be regulated by the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. Furthermore, it has been shown that transcriptional pathways involving Nrf2 and NFκB significantly interact under conditions of oxidative stress and inflammation. Because pathologic cells in RA have a higher chance of surviving, Nrf2's influence on concomitant pathologic mechanisms in the disease is explained by its interaction with key redox-sensitive inflammatory pathways. The current review not only updates knowledge about Nrf2's function in RA but also highlights the complex interactions between Nrf2 and other redox-sensitive transcription factors, which are essential to the self-sustaining inflammatory processes that define RA. This paper also reviews the candidates for treating RA through Nrf2 activation. Finally, future directions for pharmacologic Nrf2 activation in RA are suggested.



INTRODUCTION

Rheumatoid arthritis (RA), a chronic and systemic autoimmune disease with a noticeable inflammatory change in the bones and cartilages.[1] Autoantibodies such as rheumatoid factors against the Fc portion of IgG and anticitrullinated protein antibodies (ACPAs) against numerous proteins, including fibrinogen, α-enolase, vimentin, collagen type II, and fibronectin.[2] HLA-DRB1 polymorphisms, which are strongly linked to self-peptide binding and antigen presentation to autoreactive T cells, also carry a genetic risk in addition to autoantibodies.[3] Numerous immune and nonimmune cells work together to define the inflammatory microenvironment that is self-sustaining in RA.[4] These responses primarily involve the production of cytokines and the polarization of macrophages[5] and T cells[6] toward pro-inflammatory phenotypes.[7] The current understanding of RA highlights the role of stromal fibroblast-like synoviocytes (FLS) within the synovial niche, both as active contributors to the development of the pathologic synovial niche and as recipients of RA-specific cues. Research has demonstrated that in reaction to TNFα, FLS themselves release pro-inflammatory factors, such as IL-6, which mediates the crosstalk with other immune cells in the synovium, particularly supports B cells and antibody production as well as T cell survival and proliferation and differentiation toward TH17.[8] Furthermore, a transition from oxidative phosphorylation to glycolytic ATP production is initiated in FLS by the pro-inflammatory and hypoxic synovial environment. This leads to enhanced FLS survival, myeloid cell recruitment to the synovial lining layers, the production of inflammatory factors, and ultimately, bone erosion and cartilage damage.[9]

Anticytokine treatments' success was, in fact, a major advancement in the management of RA, even though the first-line treatment is still methotrexate (MTX), an antifolate drug.[10] Although the primary focus of current biologic therapies for RA is cytokine-mediated inflammation, other pathologic processes that accompany inflammation in RA must also be considered. These include increased production of reactive oxygen species (ROS) and the oxidative stress that follows, as well as modifications to redox-sensitive signaling pathways. We highlight the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which controls a broad panel of cytoprotective genes, including those involved in redox control, in the inflammatory, oxidative, and hypoxic synovial niche that characterizes RA (Figure 1). Many chronic diseases have been shown to disrupt the Nrf2 signaling pathway, which makes it a useful therapeutic target in pathologies characterized by persistent low-grade redox alterations and inflammation.[11] In place of conventional antioxidant supplements, which have been demonstrated to be effective in treating acute illnesses but not

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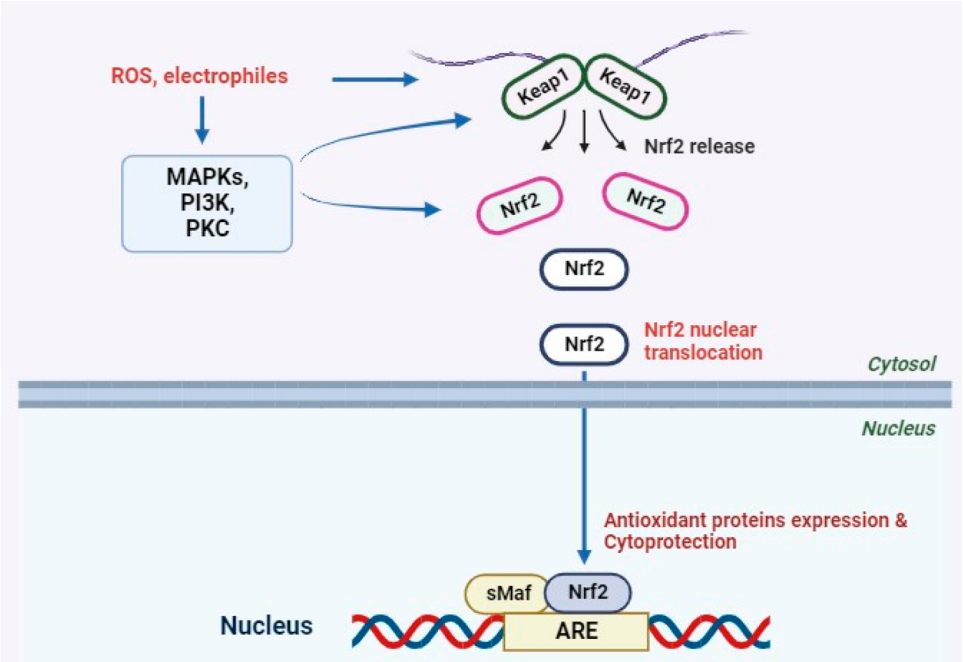

Figure 1. Nrf2 activation by reactive oxygen species (ROS) or electrophiles. Following its release from the Keap1-Cul3-RBX1 complex, Nrf2 moves into the nucleus, where it heterodimerizes with small Maf proteins (sMaf) and binds to antioxidant response elements (AREs), causing ARE-driven genes to undergo transcription.

chronic ones and may even have negative long-term effects, pharmacologic Nrf2 activation may be a safe and effective substitute.[12] Thus, we have discussed the potential advantages of targeting Nrf2 signaling pathway therapeutically for redox modulation, switching inflammation toward anti-inflammatory side and cytoprotection to alleviate the side-effects of synthetic antirheumatic drugs.

CORRELATION BETWEEN OXIDATIVE STRESS AND INFLAMMATION IN RA

Significant redox disturbances were observed at both the local and systemic levels in RA, in close conjunction with inflammation.[13] Increased ROS in RA are brought on by pro-inflammatory cytokines, danger-associated molecular patterns (DAMPs), and hypoxia in different resident synovial cell types and blood leukocytes that are drawn to the region of inflammation.[14] Overproduction of ROS in RA is caused by activation of NADPH-oxidases.[14,15] which increases the production of superoxide. The primary source of ROS in the RA synovium is the infiltrating neutrophils that possess the NOX2 isoform of the NADPH-oxidase family.[16] Activated cells produce IL-8, a chemokine that causes an intense recruitment of these cells to the inflammatory synovium.[17] TNFα primes recruited neutrophils to react forcefully to a range of stimuli, including DAMPs acting through TLRs (toll-like receptors), producing large amounts of ROS through NOX2 activation,[18] and releasing strong chemoattractants that encourage leukocyte recruitment to the synovium,[33] thereby prolonging the inflammatory processes. Numerous redox-dependent reactions that activated neutrophils produce aid in the pathogenesis of RA.[19] The inflammatory phenotype of synoviocytes in RA is shaped by mitochondrial ROS, which profoundly modify redox signaling. These mitochondrial components function as DAMPs, stimulating immune receptors and the NLRP3 inflammasome.[20] Through collagen oxidation, the local oxidative environment leads to articular damage.[13] Additionally, aberrant differentiation, growth, and survival of FLS cause release of pro-inflammatory cytokines and tissue-degrading matrix metalloproteinases that prolong joint destruction and pannus formation.[21]

It has been demonstrated that higher intracellular ROS levels in FLS, which are generated by NOX4 activation in response to TNFα and IL-17, enhance FLS migration and invasion as well as the expression of adhesion molecules and angiogenic factors.[22] Particularly in the case of RA patients with moderate disease, extracellular ROS originating from activated leukocytes infiltrating the synovium[23] can also modify the pathogenic behavior of FLS.[24] Early research noted that RA synovial intimal lining cells expressed more of the NFκB components p50 and p65 than normal synovium.[25] Increased NFκB activity was also attributed, in part, to improper TLR signaling that was sparked by host-derived ligands such as lipids, proteins and lipoproteins. The degree of RA is linked to NFκB activation in terms of bone erosion and a reduced patient response to anti-TNFα therapy.[26] It seems that in RA, NFκB maintains synoviocyte survival and inflammatory functions. Both immune and nonimmune cells express the inducible transcription factor NFκB.[27] It reacts to a range of stimuli that are important in RA, such as DAMPs (HMGB1, citrullinated histones, and S100 proteins), cytokines (TNF-α and IL-1β), and other stimuli.[28,29] In addition, NFκB stimulates the transcription of other genes that produce pro-inflammatory cytokines like IL-6, which are linked to structural and systemic bone loss in RA.[30] In RA, noncanonical NFκB activation pathway involves proteolytic processing of inactive full-length NF-κB2 (p100) into active p52 form, which translocates mainly in p52/RelB heterodimers to the nucleus, thereby promoting autoimmunity and chronic inflammation by maintaining B cell survival, differentiation, and antibody production.[31] It has also been reported that RA FLS exhibit increased PI3K/AKT activity, which explains their increased NFκB-mediated survival and decreased vulnerability to Fas-

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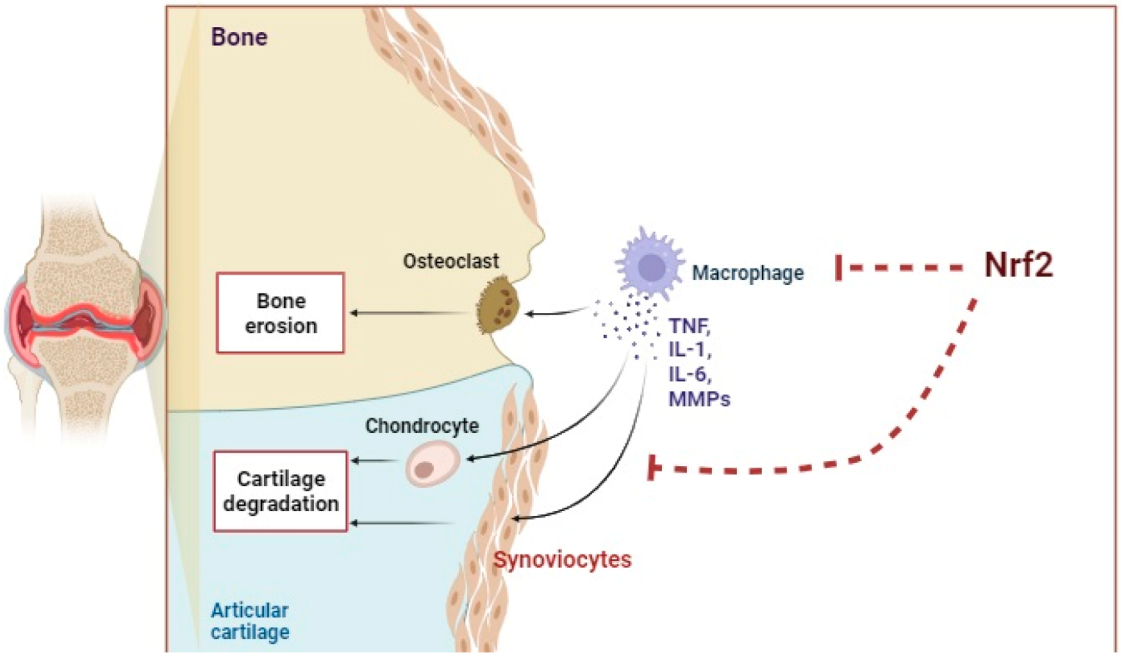

Figure 2. Regulation of the immune response in RA by Nrf2 activation.

induced apoptosis.[32] One possible explanation is that elevated ROS levels associated with RA suppress critical phosphatases, including PTEN (phosphatase and tensin homologue deleted on chromosome 10), which is the primary inhibitor of the PI3K/AKT pathway. Low concentrations of ROS are thought to activate NFκB, whereas high concentrations might have a negative impact on the transcriptional program mediated by NFκB. Under basal conditions, p50 is oxidized at the level of Cys62 in the Rel homology domain and is kept in the cytosol. Lower expression of the inducible form of cyclooxygenase occurs when high oxidative conditions, like those caused by NOX2 activation in neutrophils, occur due to oxidation and subsequent S-glutathionylation of p50 and p65, which reduces their binding to the promoter region of target genes.[33] Redox- and phosphorylation-mediated modifications, as well as dynamic, as-yet-unknown protein−protein interactions, all impact the complex transcriptional program of NFκB.

NRF2 SIGNALING PATHWAY − A NEW CONTENDER IN RA

The cytoprotective Keap1/Nrf2 pathway would most likely be activated by the elevated ROS levels that typically accompany inflammation. Nrf2, a widely distributed basic leucine zipper (bZIP) protein, forms heterodimers in the nucleus with a number of other bZIP proteins, the most well-studied of which are the small musculoaponeurotic fibrosarcoma (MAF) isoforms K, G, and F. About 250 genes contain an enhancer known as the Antioxidant Response Element (ARE), which the heterodimer binds.[34] Phase 2 detoxification and biotransformation reactions that involve the removal of xenobiotic compounds are mediated by the first discovered ARE-genes. Nrf2 is now acknowledged as the master regulator of redox homeostasis which is evident by the fact that numerous ARE genes are involved in the metabolism of glutathione, TRX, peroxiredoxin, glutaredoxin, and sulfuridedoxin. Several proteasome and autophagy genes are regulated by other cytoprotective Nrf2 target genes, which help maintain proteostasis. It has also been documented that Nrf2 functions in the activation of multiple anti-inflammatory genes and the suppression of pro-inflammatory mediators. The half-life of Nrf2 varies from 15 to 40 min, depending on the type of cell.[35] Nrf2-ECH homology (Neh) domains, a group of seven phylogenetically conserved regions, are present in it.[36] Among these, two motifs—DLG and ETGE—found in the N-terminus of the Neh2 domain bind to the primary Nrf2 regulator, the E3 ubiquitin ligase adapter Keap1 (Kelch-like ECH-associated protein 1), with varying degrees of affinity.[37,38] When Nrf2 is in its basal state, Keap1 binds to it at both the DLG and ETGE motifs, presenting it to the CUL3/RBX1 complex for ubiquitination and eventual 26S proteasome degradation.[39] Multiple extremely reactive cysteine residues found in Keap1, such as Cys151 in the BTB domain, Cys226, Cys273, and Cys288 in the IVR domain, and Cys613 at the Kelch domain, function as redox sensors.[40] It has been proposed that when Nrf2 saturates Keap1, freshly synthesized Nrf2 translocate to the nucleus and avoids degradation.[37]

Since Nrf2 regulates the transcription of multiple antioxidant genes, it plays a crucial role in diseases like RA that are highlighted by long-term oxidative stress.[41] Remarkably, Nrf2 plays a crucial role in the biosynthesis of glutathione by promoting the expression of the genes *GCLC, GCLM, GSS*, and x*CT*, which respectively encode the catalytic and modifier subunits of glutamate-cysteine ligase, glutathione synthetase, and the SLC7A11 antiporter, responsible for transportation of cystine in exchange for glutamate.[42] Additionally, Nrf2 regulates the thiol status by promoting the expression of both TRX and TRXRD1, a thioredoxin reductase that recycles reduced TRX at the expense of NADPH.[43] Moreover, Nrf2 lowers hydrogen peroxide and organic hydroperoxide levels by triggering the transcription of important antioxidant genes that code for catalase and a number of glutathione peroxidases.[44]

Cellular metabolism is significantly impacted by Nrf2's transcriptional activity. Itaconate, an anti-inflammatory metabolite produced as a byproduct of the tricarboxylic acid cycle (TCA cycle), activates Nrf2 by alkylating cysteine residues 151,

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257, 288, 273, and 297 on Keap1.[45] The type I interferon response, whose signature was demonstrated in RA, and was demonstrated to correlate with ACPA (anti-citrullinated peptide antibody), appears to be diminished by itaconate via Nrf2 activation.[46] It has been demonstrated that a deficiency in Nrf2 causes a reduction in the efficiency of oxidative phosphorylation.[47] This is coupled with a limitation in substrate availability that impairs complex I activity and increases the production of ROS within the mitochondria. Rather, glycolysis is increased.[48] Further proof comes from a functional study employing Nrf2 siRNA, which validates the function of Nrf2 on the metabolism of folate and glutamine as well as purine biosynthesis, especially in fast proliferating cells like cancer cells and FLS.[49] It has been shown that TNFα and elevated ROS levels stimulated Nrf2 expression in RA synovial tissues (Figure 2). Additionally, Nrf2 inhibition with ML385 or Nrf2 knockdown by siRNA facilitated the invasion and growth of RA FLS and the production of matrix metalloproteinases.[50] Additionally, this study demonstrated a significant inhibitory effect of Nrf2 activation with sulforaphane (SFN), suggesting that therapeutic electrophiles may be able to regulate synovitis in RA. There are concerns about the potential consequences of a persistent pharmacological activation of Nrf2, which could prolong synovitis in specific situations, given that Nrf2's role in the transition from oxidative phosphorylation to glycolysis depends on the type of cell under specific stressful conditions.[50] Research has demonstrated that constitutive Nrf2 activation in Keap1-null mice can cause postnatal death, which can be prevented by downregulating Nrf2.[51] This indicates that excessive Nrf2 activity can have negative. effects.

Findings also demonstrating the TRX system's up-regulation point to a higher level of systemic oxidative activity in RA patients' blood, which is what Nrf2-induced endogenous antioxidant mechanisms are designed to counter.[52] The Nrf2 target gene, which codes for NAD(P)H:quinone oxidoreductase (NQO1), was found to be impaired in RA patients' synovial tissue, according to an analysis of the microarray GSE39340 data set that was uploaded to the Gene Expression Omnibus (GEO) database.[53] Conversely, additional research revealed that NQO1 was activated in RA patient synovial fibroblasts following calycosin treatment, which enhanced p62 accumulation and, as a result, noncanonical Nrf2 activation.[54] The synovial tissue of a rat model of RA treated with dihydromyricetin (DMY) also showed an up-regulation of NQO1. The RA synovial tissue's aberrantly low mRNA and protein levels of Nrf2 targets heme oxygenase 1 (HO-1) and NQO1 were up-regulated by DMY in this model.[55] The solute carrier gene SLC3A2, which is regulated by Nrf2, is one of the less studied genes in RA.[56] According to one analysis, SLC3A2 transcripts were down-regulated in the blood of RA patients following MTX treatment, but up-regulated in the blood of RA patients compared to controls.[53] It has been demonstrated that sulfasalazine, a first-line treatment for RA, inhibits the cystine/glutamate antiporter.[57]

It has been demonstrated that the NFκB pathway elements NFκB1/2, RelA, and RelB play a crucial role in the type of inflammation unique to RA, and that IKK2 kinase functions as a dual modulator of arthritis, addressing both the death response and the inflammatory response. The homeostatic mechanism of NFκB and Nrf2 has been widely documented to involve a mutual transcriptional antagonistic relationship. Therefore, lipopolysaccharide (LPS)-induced NFκB activation can boost Nrf2 activity through the Ras-related C3 botulinum toxin substrate 1 (RAC1) small GTPase, which facilitates oxidative processes and β-actin-dependent cytoskeletal rearrangements. Consequently, increased Nrf2 activity can prevent RAC1-dependent NFκB activation, aiding in the resolution of inflammation.[58] When oxidative conditions are present, the Nrf2 repressor Keap1 in the cytoplasm is negatively impacted by both Nrf2 and NFκB, leading to the suppression of NFκB-mediated transcription of inflammation genes. The NFκB repressor IκBα is phosphorylated by the IKK complex, which causes the ubiquitin-proteasome to degrade it and release NFκB. The ETGE and DLG motifs needed to interact with Keap1 are present in the kinase domain of the human IKKβ subunit, and it has been proposed that Keap1 may cause IKKβ to degrade, which would activate NFκB.[59] To this end, Keap1 promotes the autophagic degradation of IKKβ by preventing the heat shock protein HSP90 from binding to it.[60] Additionally, Keap1 binding prevents TAK1 from phosphorylating IKKβ at S177 and S181, which lowers IKKβ activation.[61] The irreversible alkylation of Keap1 under extreme oxidative conditions prevents it from interacting with Nrf2 and IKKβ.[62]

GSK3β, a glycogen synthase kinase, acts independently of Keap1 to phosphorylate serine residues in the Neh6 domain of Nrf2, which is then primed for proteasomal degradation via ubiquitination by a β-TrCP/Cul1 E3 ligase complex. By controlling the activation and metabolism of synovial cells, the NRF2-targeted gene HO-1 protects joint tissues from oxidative, inflammatory, and hypoxic stress.[63] RA synovial tissue's lining and sublining layers, as well as synovial fluid, were found to have elevated levels of HO-1.[64] A portion of HO-1's anti-inflammatory effects are brought about by NFκB inhibition in the cytoplasm. The heme group is broken down by HO-1, which results in biliverdin. Biliverdin reductase then turns this bilirubin into carbon monoxide and ferrous iron, which is further broken down by Nrf2-driven ferritin induction and the activation of the ATPase Fe^{2+}-secreting pump. As proved by Li et al.'s study,[65] bilirubin prevented LPS-primed macrophages from producing TNF-α and IL-6 by reduced phosphorylating IκBα and p65, and also by caspase 1-dependent IL-1β maturation by NLRP3, AIM2, and NLRC4 inflammasomes. The CREB binding protein (CBP), a crucial coactivator that is only found in trace amounts in the nucleus, is contested by the NFκB component p65, Nrf2, and cyclic-AMP Response Element Binding Protein (CREB) in the nucleus. While CBP interacts with p65 through the KIX region of CBP, which is also in charge of binding the transcriptionally active S133-phosphorylated form of CREB,[66] CBP binds to Nrf2 through its Neh4 and Neh5 domains.[67] Furthermore, as observed in cancer cells and FLS, under conditions of extended hypoxia, CREB, NFκB, and the hypoxia-induced factor (HIF) 2 work together to promote the expression of matrix metalloproteinase 1 and consequent alterations in cell shape, migration, and invasion.[68] NFκB-p65 is observed to enhance MAFK (v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog K) associated histone deacetylase activity by recruiting histone deacetylase 3 (HDAC3) to the ARE-enriched enhancers, which further supports NFκB's inhibitory action on the transcriptional activity of Nrf2.[69] Additionally, p65 can facilitate HDAC3's association with MAFK, thereby impeding the formation of heterodimers with Nrf2.[69] Since that Nrf2-knockdown raises MAFK levels, the availability of MAFK is also regulated by Nrf2. This suggests that nuclear Nrf2 is necessary to maintain low levels of this protein in order to prevent p65 activation by acetylation.[70]

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Increased oxidative stress is likely the cause of the increased DNA damage observed in the RA synovium in both immune cells and FLS.[71] In fact, it has been noted that RA FLS overexpresses the wild-type p53 tumor suppressor (p53), which is essential for repairing DNA damage.[72] Furthermore, certain p53 mutations found in RA patients impair p53's capacity to suppress the expression of IL-6. Both p53 and Nrf2 transcriptionally regulate the p21/WAF1/CIP1/CDKN1A, which is where Nrf2 and p53 interfere. This level of interference stops the cell cycle between the G1 and S interface, allowing damaged DNA to be repaired by repair mechanisms. Nrf2 activation in RA may be combined with p53 mutations or dysfunction of repressors at the DNA level to explain the decreased levels of p21 observed in the synovium.[73,74] Nrf2 is also activated by p21/WAF1/CIP1/CDKN1A, and Nrf2 increases p21 expression through a feed-forward loop.[75] Consequently, lower Nrf2 activation through this alternative pathway is anticipated in the context of decreased p21 levels in RA, potentially having detrimental effects on redox control and anti-inflammation in RA.

PHARMACOTHERAPY OF RA VIA NRF2 SIGNALING PATHWAY

Although the number of Nrf2 activators and the corresponding patents is constantly increasing, most of these compounds have mostly stayed in the preclinical stage of development.[76] In 2013, Tecfidera (Biogen, US) was granted a license to exclusively use DMF as an oral first-line treatment for relapsing-remitting multiple sclerosis. Further research is being conducted using a more limited patient selection, despite the fact that a promising phase 3 clinical trial for type 2 diabetes mellitus and chronic kidney disease using the powerful Nrf2 activator bardoxolone methyl was initially withdrawn due to the increased risk for early onset fluid overload in patients with identifiable risk factors for heart failure.[77] With extremely encouraging results, the triterpenoid omaveloxolone is currently being studied in a phase 3 trial to treat Friedreich's ataxia (NCT02255435, EudraCT2015−002762−23). In order to identify the most promising candidates in terms of druggability and specificity for the Keap1/Nrf2 pathway as well as potential off-target effects, it is currently imperative to critically analyze the multitude of structures that demonstrate Nrf2-activating abilities in preclinical models using high-throughput and structure-based virtual screening.

MTX is still the gold standard for treating RA, but despite its proven therapeutic benefit, it is frequently stopped. The primary reasons for stopping RA treatment are toxicities to the liver, gastrointestinal tract, and kidneys, which can happen even at the low doses of MTX (15−20 mg/week), particularly in patients with comorbidities. It is anticipated that Nrf2 activation would lessen the harmful effects of xenobiotics due to its extensive cytoprotective actions linked to glutathione S-transferase and the UDP glycosyltransferase family. The Keap1/Nrf2 pathway can be activated by a variety of synthetic and natural substances.[78,79] These substances fall into two major categories: The thiol-rich Keap1 repressor contains electrophilic compounds that covalently modify critical cysteine residues. This alteration in Keap1 conformation and its interaction with Nrf2 and the CUL3/RBX1 complex results in the stabilization of newly synthesized Nrf2 molecules. Another set, peptides and small molecules known as protein−protein interaction (PPI) inhibitors prevent newly synthesized Nrf2 molecules from being directed toward Keap1-mediated proteasomal degradation. Instead, they cause the molecule to dock with the Kelch propeller of Keap1, disrupting the process and causing the transcription of ARE-genes.

Zheng Qing Feng Tong Ning is recognized as an anti-RA medication and their primary ingredient is pure alkaloid Sinomenine (SIN), which was isolated from the Chinese medicinal plant *Sinomenium acutum*.[80] In a recent report, the arthritis-suffering mice's symptoms were reduced by SIN-protected joints from destruction, suggesting that SIN's mechanism of action differs from that of MTX.[81] In synovium fibroblasts derived from RA patients, SIN significantly inhibited the production of ROS and the secretion of IL-6 and IL-33, which in turn mediated a protective effect on bone destruction and an antiarthritis effect. Moreover, in these synovium fibroblasts, SIN induced phosphorylation of p62 at Ser349 and Thr269/Ser272 to activate Keap1-Nrf2 signaling. However, mice lacking Nrf2 ($Nrf2^{-/-}$) showed a significant reduction in the antiarthritic effect of SIN. It is also known that targeting the gene p62/SQSTM1, transcription factor Nrf2 induces antioxidant response element-driven gene transcription, thereby generating a positive feedback loop.[82] Interestingly, Liao et al.[81] also discovered that in Nrf2 deficient mice compared to their wild littermates, p62 phosphorylation at Ser351 was up-regulated, suggesting that Nrf2 likely negatively regulates p62 phosphorylation at Ser351. Phosphorylation at Thr269/Ser272 was also significantly reduced. Similarly, resveratrol (RES), a natural polyphenol, activated the silent information regulator 1 (SIRT1)/Nrf2 signaling pathway, which in turn reduced the generation of ROS and FLS growth.[83] It has also been established that NFκB directly binds to the promoter of miR-29a-3p and miR-23a-3p to negatively regulate their expression. While miR-23a-3p directly targeted cullin3, miR-29a-3p had a downstream target in Keap1.

Gedunin (GDN), a limonoid that has been extracted from natural sources, such as *Azadirachta indica*. GDN prevented cytokine levels, ROS expression and mRNA and protein expression of iNOS in IL-1α-stimulated primary rheumatoid arthritis synovial fibroblasts (RASFs).[84] The results of the sip62 interference demonstrated that GDN reduced the expression of HO-1 and Keap1, as well as failed to inhibit cytokines following the sip62 interference. In a similar report, 7-deacetyl-gedunin (7-d-GDN), isolated from fruits of *Toona sinensis*, significantly reduced the expression of MMP-1/3/9/13 in RASFs, IL-6, and IL-33 in MH7A cells.[85] Additional mechanistic research revealed that 7-d-GDN induced NQO1 and HO-1, all of which were involved in the suppression of oxidative stress. Sequestosome 1 (SQSTM1, p62) was elevated by 7-d-GDN, which down-regulated Keap1, leading to Nrf2 cytoplasm accumulation and subsequent translocation into the nucleus.

Oleuropein (OL), an olive tree secoiridoid and its peracetylated derivate (Per-OL) was studied on collagen-induced arthritis (CIA) murine model.[86] Both Per-OL and OL diets ameliorated serum collagen oligomeric matrix protein (COMP), MMP-3 and pro-inflammatory mediators. In mice fed OL and Per-OL diets, there was a significant down-regulation of MAPK and NFκB activations and an up-regulation of Nrf2 and HO-1 protein expressions. Another important naturally occurring substance present in the stems and roots of *Alangium chinense* is salicin, a prodrug form of acetyl salicylic acid (Aspirin). In IL-1β-induced RA-FLSs, salicin dramatically decreased cell viability, cytokines, TNF-α, IL-6, and matrix metalloproteinases-1/-3 expression.[87] It also inhibited the production of ROS and phosphorylation of p65. Furthermore,

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in IL-1β-induced RA-FLSs, salicin decreased ROS production and increased HO-1 expression and Nrf2 nuclear translocation. Salicin suppressed the oxidative damage indexes in addition to lowering the clinical score, inflammatory infiltration, and synovial hyperplasia in vivo in collagen-induced arthritis. Epigallocatechin 3-gallate's (EGCG) anti-inflammatory properties were demonstrated by Ahmet Karatas and colleagues.[88] Measurements were made of a number of parameters, including serum TNF-α, IL-17 levels, antioxidants, synovial inflammation, and cartilage bone degradation in collagen-induced arthritis model. The groups administered with EGCG showed improvements in a number of parameters, demonstrating the antiarthritic effect of EGCG through cytokine and redox balance via Nrf2 signaling pathway.[88]

S-propargyl-cysteine (SPRC, also known as ZYZ-802) was investigated for protection against RA.[89] In IL-1β-induced MH7A, MMP-9 expression and activity, ROS generation, and inflammatory mediator expression were all attenuated by SPRC in a concentration-dependent manner. Furthermore, SPRC prevented MH7A cells from migrating and invading through IL-1β. They also discovered that SPRC significantly increased the expression of HO-1, which was linked to the breakdown of Keap1 and the nuclear translocation of Nrf2. This effect was explained by the sulfhydrylation of Keap1's cysteine residue. A novel synthetic compound called furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) has been shown to have anti-inflammatory properties through the inhibition of cyclooxygenase-2 (COX-2). By enabling Nrf2's nuclear translocation, FPP-3 increased the expression of several antioxidative enzymes, such as NQO1, GCL, and HO-1.[90] The inducibility of antioxidant proteins, like HO-1, was eliminated in murine fibroblasts lacking Nrf2. FPP-3 treatment in mouse fibroblasts reduced the upregulation of NFκB-driven reporter gene expression caused by lipopolysaccharide due to antioxidative capacity.

A latest report demonstrate how effectively Nrf2 activation reduces arthritis in SKG mice, that receive zymosan A injections to induce T cell-mediated autoimmune arthritis. We discovered that genetic Nrf2 activation through the knockdown of Keap1, suppressed arthritis in SKG mice by causing the expression of antioxidant enzymes and preventing the pro-inflammatory mediator's expression.[91] Furthermore, oral administration of CDDO-Im, a representative chemical inducer of Nrf2, prevented and treated SKG mouse arthritis in a way that was dependent on Nrf2.

Chrysotherapy, the use of gold compounds, is primarily utilized in the management of RA. They are used because they have few adverse effects and primarily suppress the immune system. A decrease in inflammatory mediators, such as monocytes, IL-6, and IL-1β, was noted by Costa et al.[92] in their investigation of the Au-nanospheres of methotrexate for the treatment of RA. Using antirheumatic Gold(I) Compounds, Kataoka and colleagues showed how Nrf2/Small Maf, heterodimeric transcription factors with a basic leucine zipper region, initiates antioxidative stress in cellular components and genes.[93] Auranofin, 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranosato-S-[triethylphosphine] gold, is an antirheumatic medication that contains gold(I) and has anti-inflammatory properties.[94] The mechanistic studies showed reduced Nrf2 degradation by inducing the dissociation of the Nrf2/Keap1 complex. Furthermore, treatment with auranofin triggered the expression of iNOS and activated cellular Rac1. NSC23766, an inhibitor of Rac1, prevented the induction of iNOS, Nrf2 activation, and HO-1 expression. Treatment with auranofin increased the phosphorylation of MAPKs, and partial inhibition of Nrf2 activation was observed with MAPK inhibitors.

CONCLUSION

Arguments in support of RA adjunctive therapy targeting the Nrf2 pathway are persistent. Pharmacologic activation of NRF2 is anticipated to be advantageous for conventional antirheumatic therapies because of its anti-inflammatory and antioxidant properties, as well as its ability to protect organs impacted by long-term synthetic drug treatment. As a result, the validity of the suggested biochemical and molecular networks—which would be extremely helpful for developing and testing novel therapies—was limited. The majority of the data on Nrf2 activators were collected in preclinical settings, which do not accurately mimic the complex pathologic mechanisms of RA. Using improved *in silico*, *in vitro*, and animal models, a systematic reevaluation of the existing electrophilic and PPI inhibitor compounds in terms of efficacy and side-effects should be carried out in order to identify the most promising candidates to be advanced in the drug development pipeline for the treatment of RA.

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Notes

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