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FROM PATHWAYS TO THERAPIES: THE ROLES OF UBIQUITINATION AND MAPK SIGNALLING IN RHEUMATOID ARTHRITIS

Hong Chen[1,2], Sha Liu[1], Zhang Yu[1], Der Jiun Ooi[2*], and Hua Wei[3*]
1 Department of Rheumatology and Immunology, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China.
2 Department of Preclinical Sciences, Faculty of Dentistry, MAHSA University, Bandar Saujana Putra, 42610 Jenjarom, Selangor, Malaysia.
3 Department of General Practice, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China.

Corresponding Author(s): Dr. Der Jiun Ooi, E-mail: djooi@mahsa.edu.my. Professor Hua Wei, E-mail: weihch123456@163.com.

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation, synovial hyperplasia, and joint destruction. Despite advances in treatment, identifying effective therapeutic targets remains a significant challenge. Recent research has highlighted the roles of ubiquitination and the mitogen-activated protein kinase (MAPK) signalling pathway in RA pathogenesis. The post-translational ubiquitination modification governs immune responses, synovial fibroblast proliferation, and apoptosis by modulating protein stability and function. Concurrently, MAPK signalling drives inflammatory cytokine production and contribute to joint damage through its key components including ERK, JNK, and p38. Notably, Ubiquitin D (UBD) that acts like ubiquitin exarcebates RA progression by activating the p38 MAPK pathway, positioning it as a promising therapeutic target. Emerging evidence suggests that dual-target strategies that simultaneously modulate both ubiquitination and MAPK signalling may synergistically suppress inflammation and aberrant cell proliferation, which offers new avenues for RA management. This review summarizes recent mechanistic insights and underscores the need for further research to unravel the crosstalk between these pathways and evaluate their clinical potential. By bridging molecular mechanisms with therapeutic applications, this work aims to inform future strategies for improving RA treatment outcomes.

Keywords: rheumatoid arthritis; ubiquitination; MAPK signaling; therapeutic strategies; inflammation; signal transduction

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease that primarily affects the joints, resulting in synovial hyperplasia, pannus formation, cartilage destruction, and various systemic complications [1, 2]. Beyond joint damage, RA significantly increases the risk of cardiovascular, pulmonary involvement, and infections. All of which contribute to reduced quality of life and shortened life expectancy in affected individuals [3]. Clinically, RA typically presents with symmetrical joint pain, swelling, and stiffness, involving both small and large joints [4]. Globally, the prevalence of RA is approximately 0.46%, with a higher incidence in women than in men, with a female-to-male ratio of 3:1[5,6].

Over the past decades, substantial clinical advances have been made in RA management, particularly through the development of Disease-Modifying Anti-Rheumatic Drugs (DMARDs). These include conventional synthetic DMARDs (e.g. methotrexate, hydroxychloroquine, and sulfasalazine), biological DMARDs (e.g. tumour necrosis factor (TNF)-α inhibitors, TNF receptor inhibitors, interleukin (IL)-6 inhibitors, IL-6 receptor inhibitors, B-cell depletion antibodies, and co-stimulation molecule inhibitors), as well as targeted synthetic DMARDs (e.g., pan-JAK and JAK1/2 inhibitors). These agents have demonstrated efficacy in reducing disease activity and limiting joint damage [7-9].

Despite these advancements, treatment challenges remain. A subset of patients remains refractory to existing therapies, highlighting the ongoing need for novel molecular targets and therapeutic strategies [10]. At the molecular level, RA is driven by complex signalling networks involving the overproduction of pro-inflammatory cytokines and dysregulation of intracellular signalling cascades.

Emerging evidence suggests that UBD contributes to RA progression by activating the p38 MAPK pathway, thereby promoting synovial inflammation, cytokine secretion, and fibroblast-like synoviocyte (FLS) proliferation [11]. This mechanistic link positions UBD as a compelling example of how ubiquitination can directly regulate MAPK signalling in RA. Consequently, therapeutic strategies that simultaneously target both ubiquitination pathways and MAPK signalling may offer synergistic benefits by disrupting key pathological feedback loops. This dual-modulation approach has the potential to more effectively reduce inflammation, control aberrant synovial cell activity, and preserve joint integrity in RA patients.

This review provides a comprehensive overview of the mechanistic roles of ubiquitination and the MAPK signalling pathway in RA. Special attention is given to their crosstalk, pathophysiological relevance, and therapeutic potential, with UBD highlighted as a representative target bridging both pathways.

2. Pathological Mechanisms of Rheumatoid Arthritis

RA is a multifactorial autoimmune disorder. Its aetiology is shaped by a combination of genetic predisposition, environmental exposure, and immune dysregulation. Among genetic factors, human leukocyte antigen (HLA) class II alleles have been most strongly linked to RA susceptibility [12, 13]. Environmental triggers such as smoking, occupational exposures, and periodontal disease further elevate RA risk [14, 15]. More recently, infections and gut microbiota dysbiosis have garnered increasing attention, as they appear to implicate in RA pathogenesis through influencing immune regulation and autoantibody generation [16-18].

2.1 The Role of Microbiota and Mucosal Immunity

Several studies highlight the role of mucosal surfaces in RA initiation. One study reported that individuals with periodontitis have a 69% increased risk of developing RA compared to those without [19]. Dysbiosis of the gut microbiota in RA patients has also been observed, including the enrichment

Fusobacterium nucleatum. This pathogen exacerbates joint inflammation through the release of outer membrane vesicles containing the virulence factor FadA. These vesicles penetrate joint tissues and activate localised inflammatory responses by acting on synovial macrophages, eventually triggering downstream inflammatory mediators such as Rab5a GTPase and YB-1 [20].

Disrupted host-microbiota interactions at mucosal sites may serve as upstream modulators of systemic immune dysregulation in RA [21]. Nonetheless, major research gaps persist. The precise mechanisms by which microbial imbalances modulate RA-related immune responses remain elusive [22]. More evidence is needed to evaluate whether microbiota-targeted therapies can meaningfully alter disease onset or progression [23].

2.2 Autoantibodies and Immune Cell Involvement

The immunopathogenesis of RA is marked by an aberrant autoimmune response characterised by the abnormal activation of autoantibodies and involvement of various immune cells such as T cells, B cells, and macrophages. These cells secrete inflammatory cytokines and chemokines that lead to joint damage and loss of function [24-26]. Specifically, T cells stimulate B cells to produce autoantibodies, while also activating macrophages and amplifying the inflammatory cascade [27]. Two hallmark autoantibodies, namely rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP), form immune complexes that promote immune cell recruitment and synovial inflammation [28-30].

2.3 Cytokine Networks in RA Pathogenesis

Proinflammatory cytokines including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) are central to RA pathophysiology. These cytokines exacerbate joint damage by activating osteoclasts, driving inflammatory cell recruitment, and sustaining synovial hyperplasia [31-33]. Collectively, synovial cell proliferation, angiogenesis, and immune cell infiltration lead to tissue remodeling processes that underlie bone erosion and cartilage degradation [24, 34].

2.4 Fibroblast-like Synoviocytes and Joint Destruction

The inflammatory synovial microenvironment in RA drives fibroblast-like synoviocytes (FLS) to undergo pathological transformation, adopting an aggressive, tumour-like phenotype that plays a central role in disease progression. These activated FLSs invade and degrade cartilage and bone through excessive production of matrix metalloproteinases [35]. Alongside infiltrating macrophages and T cells, they secrete pro-inflammatory cytokines and chemokines that recruit and activate immune cells. They demonstrate resistance to apoptosis, creating a self-perpetuating cycle of inflammation and joint destruction [36]. As illustrated in Figure 1, this process occurs in concert with infiltrating macrophages and T cells, forming a complex cellular network that drives RA pathogenesis.

At the molecular level, FLS activation is mediated through multiple signalling pathways, most notably the nuclear factor kappa B (NF-κB) pathway, which serves as a master regulator of inflammation and bone erosion in RA [37]. The pathological RA synovium undergoes dramatic remodelling that is characterized by synovial cell hyperplasia and abnormal proliferation, extensive angiogenesis supporting pannus formation, dense infiltration of immune cells and activated fibroblasts, as well as uncontrolled production of inflammatory mediators (IL-6, IL-1β) and tissue-destructive enzymes [38, 39]. Notably, TNF-α not only enhances FLS proliferation in vitro but also promotes their survival and invasive capacity. This establishes a critical feedback loop that sustains synovitis [39]. This multifaceted involvement of FLSs in both structural damage and immune dysregulation makes them a prime therapeutic target in RA.

2.5 Targeting FLS: A Therapeutic Opportunity

Given their central role in disease progression, FLSs represent an attractive therapeutic target. The antimalarial drug artesunate has been shown to inhibit the migration and invasiveness of FLS by suppressing the expression of matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9. MMPs are a class of enzymes that play a crucial role in extracellular matrix remodelling by degrading various extracellular matrix components like collagen, elastin, and proteoglycans [40]. Targeting such mechanisms may improve clinical outcomes by limiting tissue damage.

Altogether, advancing understanding of the pathological interplay between immune cells, cytokines, and FLSs is vital for identifying novel molecular targets. This foundation supports the exploration of ubiquitination and MAPK signalling as key regulatory networks that influence RA progression and treatment responsiveness.

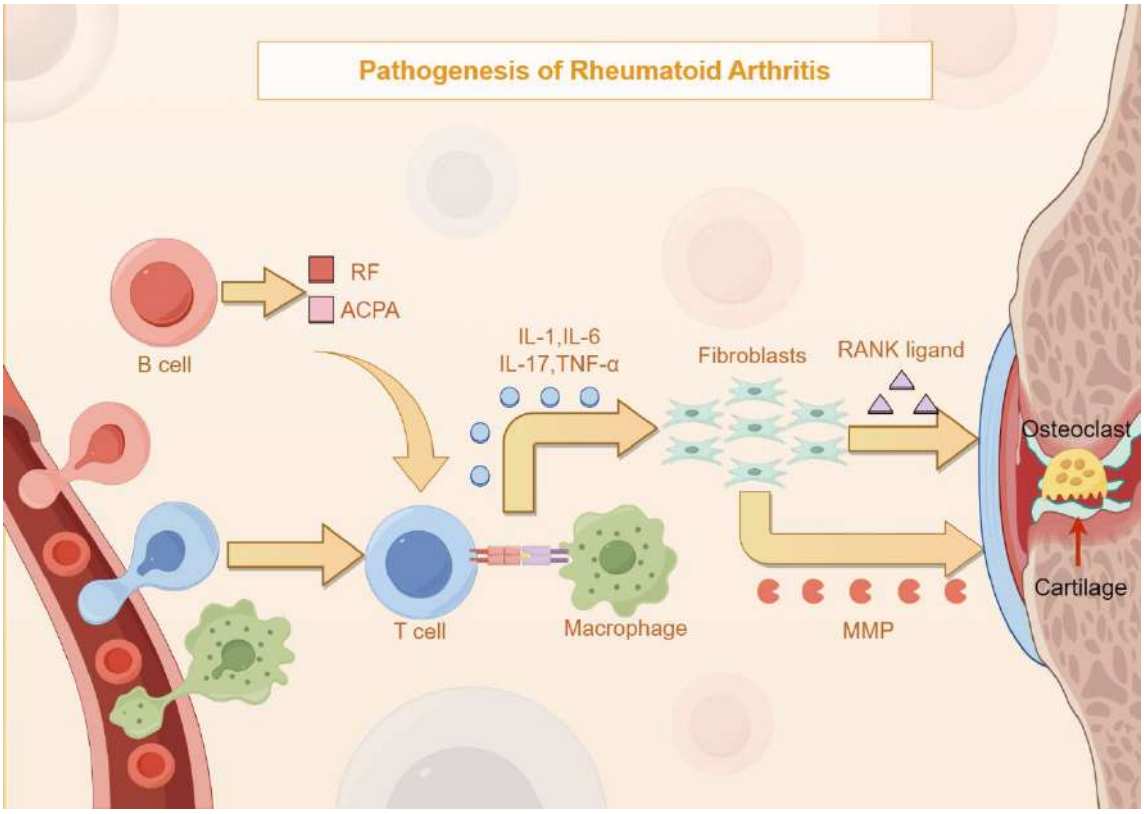

Figure 1: Pathological Mechanisms of Rheumatoid Arthritis

This figure illustrates the key cellular and molecular events involved in the pathogenesis of RA. B cells produce autoantibodies such as RF and ACPA, which contribute to immune complex formation and inflammation. Activated T cells and macrophages release pro-inflammatory cytokines including IL-1, IL-6, IL-17, and TNF-α, which further stimulate fibroblasts in the synovial membrane. These fibroblasts produce MMPs that degrade cartilage and express RANK ligand, promoting osteoclast differentiation and activation. The resulting osteoclast-mediated bone resorption and cartilage destruction are hallmarks of joint damage in RA (Image prepared using Figdraw; ID: iaiywf524b).

RA: Rheumatoid Arthritis, RF: Rheumatoid Factor, ACPA: Anti-Cyclic Citrullinated Peptide Antibody, MMP: Matrix Metalloproteinases, RANK ligand: Receptor Activator of Nuclear Factor-κB Ligand

3. The Role of the MAPK Signalling Pathway in RA

The MAPK signalling pathway is a key intracellular cascade implicated in the pathogenesis of RA, comprising three principal subfamilies: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK [41, 42]. These kinases are activated by specific external stimuli such as pro-inflammatory cytokines (e.g., TNF-α, IL-6), growth factors (e.g., FGF, EGF), and stress signals. As illustrated in Figure 2, these upstream signals activate MAPK cascades that lead to the phosphorylation of ERK, JNK, and p38. These events result in altered gene expression, inflammatory cells activation, and synovial proliferation that ultimately contribute to joint destruction [43, 44].

Among the three branches, ERK is most closely associated with synovial cell proliferation, while JNK and p38 MAPK are key mediators of inflammation. In RA, ERK hyperactivation promotes the proliferation of synovial cells, thereby exacerbating inflammation and joint pathology [45, 46]. Silencing of the long non-coding RNA NEAT1 has been shown to reduce ERK1/2 phosphorylation, correlating with attenuated synovial proliferation and inflammation [47]. Given its pivotal role in RA progression, the MAPK pathway has since emerged as a promising therapeutic target. Several pharmacological agents and natural compounds have demonstrated the ability to modulate MAPK signalling components and offer novel avenues for treatment.

4. Therapeutic Agents Targeting the MAPK Signalling Pathway in RA

Multiple natural compounds and pharmacological agents have been investigated for their ability to modulate MAPK activity and alleviate RA symptoms. Tubson-2 decoction is a traditional Mongolian herbal remedy. It has shown therapeutic efficacy to alleviate RA symptoms and osteoporosis in collagen-induced arthritis (CIA) models. One of its major active components, isochlorogenic acid A, exerts anti-inflammatory and bone protecting effects by modulating the IL-17/MAPK pathway. Specifically, it downregulates pro-inflammatory cytokines including IL-17A, TNF-α, and IL-1β, as well as IL-17 receptor and p38 MAPK, while upregulating anti-inflammatory cytokine IL-10. These changes are indicative of targeted suppression of the IL-17/MAPK axis, resulting in reduced inflammation and joint damage [48].

Naringin is a flavanone glycoside found naturally in citrus fruits. This water-soluble bitter flavonoid has exhibited anti-inflammatory and anti-proliferative effects in RA. In vitro and in vivo studies showed that naringin inhibits the phosphorylation of MAPK proteins (p-ERK, p-JNK, p-p38) and components of PI3K/Akt cascades, while enhancing apoptosis in FLS. This dual regulation suppresses the invasive behaviour of RA-FLS and reduces pro-inflammatory cytokine

production [49].

Shikonin is a natural naphthoquinone pigment isolated from the roots of the Lithospermum erythrorhizon plant. It has a long history of use in traditional medicine for its anti-inflammatory, antibacterial, anticancer, and antiviral properties. Studies have reported the multi-target anti-rheumatic effects of shikonin. It activates AMPK and inhibits Mechanistic Target Of Rapamycin/Unc-51 Like Autophagy Activating Kinase 1 (mTOR/ULK-1) signalling to induce autophagy and apoptosis in RA-FLS [50]. Additionally, shikonin downregulates PI3K/Akt and MAPK (ERK, JNK, p38) pathways which further suppress angiogenesis and inflammatory cytokine production [25]. Studies have also reported that shikonin inhibits Wingless-type/β-catenin (Wnt/β-catenin) signalling which led to disruption of mitochondrial energy metabolism-induced apoptotic cell death [51, 52]. Collectively, shikonin acts through multiple signalling axes to attenuate angiogenesis, mitochondrial metabolism, and inflammatory responses.

Fluvastatin sodium (FVS) is widely recognised as a lipid-lowering agent through its inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase. However, it also exhibits significant anti-inflammatory and immunomodulatory properties in RA. In an adjuvant-induced arthritis model, transdermal administration of FVS markedly attenuated joint swelling, synovial inflammation, and cartilage destruction. All of these effects are linked to suppressed p38 MAPK phosphorylation, as well as reduced TNF-α and IL-6 expressions [53]. Moreover, in a transgenic rat model, FVS demonstrated additional therapeutic potential by upregulating Rho GTPase-activating protein 12, which inhibited Ras homolog family member GTPase activity and consequently supressed synovial cell migration and inflammation [54].

Resveratrol is a natural stilbenoid. It is a type of polyphenol produced by plants in response to stress, injury, or fungal infections. The compound can be found in various plants, including grapes, berries, and peanuts. Well recognised for its antioxidant and anti-inflammatory properties, resveratrol exerts similar multi-target therapeutic effects in RA. First, it activates sirtuin 1 (SIRT1) while concurrently inhibiting both NF-κB and MAPK signalling pathways, leading to significant reductions in key inflammatory cytokine production including TNF-α, IL-1β, and IL-6 in the RA microenvironment [55]. Second, it alleviates RA-associated interstitial lung disease by enhancing lysosomal function through the protein kinase B/transmembrane protein 175 (Akt/TMEM175) pathway and suppressing expression of fibrotic markers TGF-β1 and α-SMA in lung tissue [55]. Third, resveratrol inhibits the abnormal synovial angiogenesis by targeting the transcription 3/hypoxia-inducible factor 1/vascular endothelial growth factor (STAT3/HIF-1α/VEGF) axis, thereby disrupting the hypoxic-inflammatory cycle that perpetuates joint damage [56]. These complementary actions of reducing inflammation, protecting against extra-articular complications, and inhibiting pathological angiogenesis essentially highlight resveratrol's potential over current single-target therapies, particularly for patients with refractory disease or systemic manifestations.

Kinsenoside is an active glycoside isolated from plants of the Anoectochilus genus with a broad range of pharmacological functions. This natural compound exhibits significant therapeutic potential in arthritis through its dual immunomodulatory and chondroprotective properties. Mechanistically, it suppresses the NF-κB/MAPK signalling pathway which plays a central role in inflammatory responses. Concomitantly, it promotes the phenotypic shift of macrophage repolarization from proinflammatory M1 to anti-inflammatory M2 states which effectively modulates the immune microenvironment. These mechanisms result in downregulation of critical inflammatory markers such as inducible nitric oxide synthase (iNOS), IL-1β, and TNF-α, while maintaining chondrocyte viability and protecting articular cartilage from degradation. The compound's multifaceted efficacy has been consistently demonstrated across both in vitro cellular and in vivo animal studies of arthritis [57]. This unique combination of properties makes kinsenoside particularly promising for the

development of novel arthritis treatments that target disease progression at multiple levels.

As a naturally occurring flavonoid glycoside derived from Iridaceae species, tectoridin has also emerged as a promising anti-rheumatic agent through its multi-target modulation of inflammatory signalling pathways. This natural compound exerts its anti-rheumatic effects through simultaneous modulation of Toll-like receptor 4/NOD-like receptor family pyrin domain containing 3/NF-κB (TLR4/NLRP3/NF-κB) and MAPK signalling pathways. Molecular investigations revealed that tectoridin effectively inhibits nuclear translocation of NF-κB p65 mitogen-activated protein kinase (p65) while downregulating the expression of both TLR4 and NOD-like receptor family pyrin domain containing 3 (NLRP3) components. Cumulatively, these led to reduced cytokine production in TNF-α-stimulated RA-FLS [58]. Furthermore, tectoridin exhibits potent synovium-protective properties by specifically inhibiting phosphorylation of ERK, JNK, and p38 MAP kinases. This coordinated action leads to reduced synovial hyperplasia, decreased inflammatory mediator release, and ameliorated joint pathology, as demonstrated using CIA models [59]. The compound's ability to concurrently target multiple nodes in RA pathogenesis, including both innate immune receptors (TLR4/NLRP3) and downstream signalling effectors (NF-κB/MAPK), distinguishes it from conventional single-target therapies. These findings collectively support tectoridin's potential as a mechanism-based therapeutic strategy that addresses both the inflammatory and destructive components of rheumatoid arthritis.

The natural polyphenol magnolol isolated from Magnolia officinalis also demonstrates potent anti-arthritic effects through its multimodal mechanisms of action. This natural compound exerts potent anti-arthritic effects by simultaneously targeting inflammatory and oxidative pathways in RA pathogenesis. At the molecular level, magnolol effectively suppresses TLR4-mediated MAPK signalling by inhibiting phosphorylation of of key kinases including ERK, JNK, and p38, leading to marked reductions in pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. Complementing these immunomodulatory effects, magnolol also addresses the oxidative stress component of RA by upregulating critical antioxidant defence systems, particularly superoxide dismutase and glutathione activity [60]. This dual action, suppressing inflammatory cascades while enhancing cellular antioxidant capacity, enables magnolol to disrupt the self-perpetuating cycle of inflammation and oxidative damage that drives RA progression.

In summary, the MAPK signalling pathway orchestrates key pathological events in RA, including synovial proliferation, inflammation, and tissue destruction. Targeting this pathway with natural compounds provides promising multi-target therapeutic strategies.

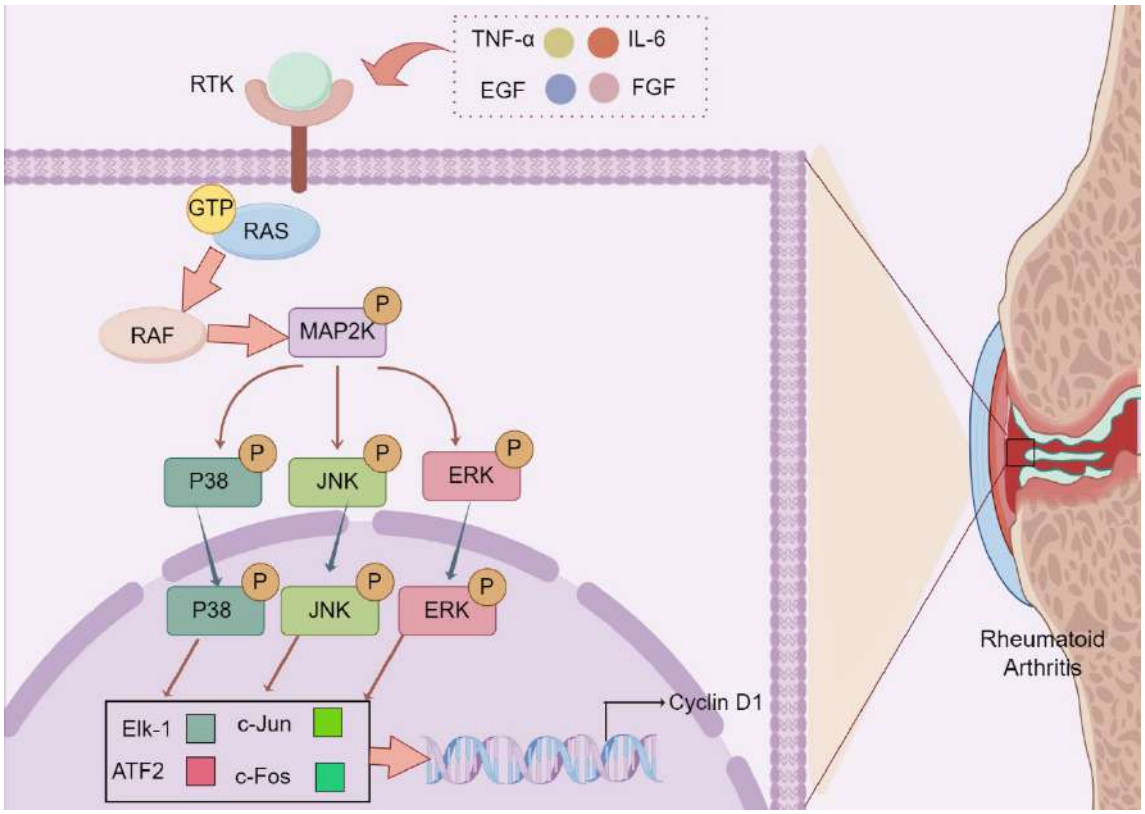

Figure 2: Activation of the MAPK Signalling Pathway Promotes RA Progression

This figure illustrates how external stimuli such as TNF-α, IL-6, EGF, and FGF activate the MAPK signalling pathway through RTKs. Signal transduction begins with the activation of RAS and RAF, leading to the phosphorylation of MAP2K. This activates the downstream MAPK branches—ERK, JNK, and p38—which translocate to the nucleus and phosphorylate transcription factors such as Elk-1, c-Jun, ATF2, and c-Fos. These events drive the expression of genes like cyclin D1, contributing to synovial cell proliferation, inflammation, and joint destruction characteristic of rheumatoid arthritis (Image prepared using Figdraw; ID: putsi5bf3b).

RTK: Receptor Tyrosine Kinase, RAS: RAt Sarcoma virus protein, RAF: RAF Protein Kinase, MAP2K: Mitogen-Activated Protein Kinase Kinase, JNK: c-Jun N-terminal Kinase, P38: P38 Mitogen-Activated Protein Kinase, ERK: Extracellular Signal-Regulated Kinase, ATF2: Activating Transcription Factor 2

Table 1: Roles of selected drugs and phytochemicals involved in the MAPK signalling pathway in RA.

Drugs/Phytochemical	Mechanism of Involvement in MAPK Signalling Pathway	Role in RA	Reference
Tubson-2 decoction (isochlorogenic acid A)	Regulates IL-17/MAPK signalling pathway	Alleviates RA symptoms and inflammation-induced osteoporosis	[48]
Naringin	Inhibits PI3K/Akt and MAPK (ERK, JNK, p38) signalling pathways	Suppresses inflammation and matrix metalloproteinases production; Promotes RA-FLS apoptosis	[49]
Shikonin	Inhibits PI3K/Akt and MAPKs (ERK, JNK, p38) signalling pathways	Exhibits anti-angiogenic and pro-apoptotic effects	[25, 42]
Fluvastatin Sodium (FVS)	Suppresses p38-MAPK signalling pathway	Reduces joint inflammation and cartilage damage in RA models	[53]
Resveratrol	Downregulates JNK, ERK, and p38 MAPK signalling pathways	Attenuates cytokine production and disease severity; Inhibits angiogenesis	[55, 56]
Kinsenoside	Inactivates NF-κB/MAPK signalling pathway	Induces macrophage M2 polarization; Protects chondrocytes	[57]
Tectoridin	Inhibits ERK, JNK, and p38 MAPK phosphorylation	Reduces synovial inflammation and hyperplasia	[58]
Magnolol	Inhibits TLR-4 mediated MAPK signalling pathway	Suppresses pro-inflammatory cytokines and oxidative stress	[60]

5. The Role of Ubiquitination in Rheumatoid Arthritis

The ubiquitin-proteasome system represents a sophisticated regulatory network that profoundly influences rheumatoid arthritis pathogenesis through its control of protein stability and function. Being a highly dynamic, enzyme-catalysed post-translational modification, ubiquitination regulates cellular and immune homeostasis through structurally and functionally distinct polyubiquitin signals [61]. This process involves the covalent attachment of ubiquitin molecules to target proteins through the coordinated action of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3). Collectively, these cascades of enzymatic activities modulate protein stability, localization, and function [62, 63]. Through structurally diverse polyubiquitin chains, ubiquitination orchestrates a range of biological processes such as cell cycle progression, apoptosis, immune responses, and intracellular transport [62]. Of particular relevance to RA, ubiquitination and ubiquitin-editing enzymes play crucial roles in regulating signalling cascades downstream of cytokine, pattern recognition, and lymphocyte receptors, especially the NF-κB pathway [64].

Aberrant ubiquitination contributes to multiple pathological features of RA, including immune dysregulation, synovial hyperplasia, and sustained inflammation. Central to this process is the E3 ubiquitin ligase STIP1 homology and U-box containing protein 1 (STUB1) which promotes the non-degradative ubiquitination of the aryl hydrocarbon receptor (AHR). This disrupts the critical balance between pro-inflammatory Th17 cells and regulatory T cells (Tregs), thereby promoting autoimmune inflammation [65]. As depicted in Figure 3, ubiquitination-mediated regulation extends beyond immune cell polarization to directly influence synovial pathology, modulating both FLS proliferation and apoptotic resistance.

As a deubiquitinating enzyme, Ubiquitin-specific protease 2 (USP2) exacerbates RA progression by removing ubiquitin chains from TRAF2 to sustain FLS proliferation and inflammatory signalling [66]. Conversely, the E3 ubiquitin ligase tripartite motif-containing protein 32 (TRIM32) promotes TRAF2 activation by promoting K63-linked polyubiquitination, which boosts proinflammatory cytokine production in the synovium [67]. These opposing yet complementary mechanisms that involve ubiquitin removal by USP2 versus specific ubiquitin chain formation by TRIM32 further exemplify the finely tuned yet dysregulated ubiquitin-mediated control in RA.

Building on these ubiquitination mechanisms, ubiquitin-like modifier activating enzyme 1 (UBA1) serves similar activities as the crucial initiating enzyme in the ubiquitin-proteasome cascade. This primary E1 enzyme catalyses the ATP-dependent activation of ubiquitin, forming a high-energy thioester bond that enables subsequent transfer to E2 conjugating enzymes [68]. This initial step is fundamental to all downstream ubiquitination events, establishing UBA1 as a master regulator of protein degradation pathways.

Other modulators, such as Midline-1 (Mid1), ZNRF3, and TNFAIP3 (A20), also influence disease progression by regulating Treg stability, Wnt signalling, or RANK-induced osteoclastogenesis. Notably, USP5, a DUB overexpressed in RA-FLS, enhances proinflammatory cytokine expression via NF-κB p65 activation, while its knockdown mitigates FLS aggressiveness. Collectively, these findings illustrate how aberrant ubiquitination drives inflammatory responses, immune imbalance, and joint damage in RA. Understanding these mechanisms provides valuable insights into RA progression and opens new avenues for targeted therapeutic strategies.

6. Therapeutic Agents Targeting the Ubiquitination in RA

Building on the mechanistic insights into ubiquitination pathways, several therapeutic agents have been investigated for their potential to modulate this system and mitigate RA pathogenesis. One such agent is auranofin, a gold-based compound historically used to treat RA.

Auranofin binds directly to the UBA1's ubiquitin-fold domain, which is an essential region for interactions with E2 conjugating enzymes. This binding promotes trans-thioesterification, which accelerates ubiquitin transfer and subsequent proteasomal degradation of misfolded endoplasmic reticulum (ER) proteins. Unlike conventional inhibitors, auranofin augments UBA1 activity, thereby alleviating ER stress and exerting anti-inflammatory effects in RA models [69].

Another emerging regulator is the dopamine D3 receptor (D3R) that is predominantly expressed on mast cells. D3R expression inversely correlates with RA severity. Upon activation, D3R promotes ubiquitination and lysosomal degradation of Toll-like receptor 4 (TLR4), suppressing the TLR4/NF-κB signalling cascade and reducing production of pro-inflammatory cytokines like TNF-α and IL-6. In contrast, D3R deficiency impairs TLR4 degradation, exacerbates inflammation, and worsens arthritis severity. These findings underscore the potential of D3R as a potential immunomodulatory target that exploits the ubiquitin-lysosomal pathway [70].

Natural compounds have also shown promising activity. β-Indole-3-acetic acid (IAA), a plant-derived molecule containing an indole ring, alleviates RA symptoms by reducing Foxp3 ubiquitination via activation of the AhR–TAZ–Tip60 complex. This complex stabilises Foxp3 through acetylation, promoting regulatory T cell (Treg) differentiation and maintaining immune homeostasis. The loss of therapeutic effect in AhR-deficient models confirms the specificity of this pathway, highlighting IAA's potential to modulate Treg-related ubiquitination in RA [71,72].

Menthone is a natural monoterpene ketone derived from Mentha species. The compound has also been identified to exhibit anti-inflammatory effect in RA by modulating protein ubiquitination. Specifically, menthone promotes K48-linked polyubiquitination of Tyrosine kinase 2 (Tyk2). This leads to its proteasomal degradation and subsequent suppression of STAT1/STAT2 phosphorylation, thereby reducing IFN-I-induced cytokines such as CXCL10 and IRF7 in synovial tissues. The net effect is reduced joint swelling and structural preservation [73].

The deubiquitinating enzymes (DUBs), previously identified as upregulated in RA-FLS, also represent a viable therapeutic target. Ubiquitin-specific protease 5 (USP5) knockdown markedly suppresses RA-FLS proliferation, migration, and invasion, while promoting apoptosis. In contrast, USP5 overexpression enhances inflammatory responses in RA-FLSs by upregulating key proinflammatory cytokines, including TNF-α, IL-6, and IL-1β through NF-κB activation. Phosphorylation and nuclear translocation of NF-κB p65 in turn activates downstream inflammatory gene transcription that further aggravates synovial inflammation and tissue destruction in RA. These findings position USP5 inhibition as a strategy to dampen synovial hyperplasia and joint damage [74].

Similarly, lentivirus-mediated silencing of ZNRF3, an E3 ubiquitin ligase, reduces the expression of inflammatory mediators such as TNF-α, IL-1β, and IL-6, which subsequently alleviates joint damage in arthritis models [76]. Another crucial ubiquitin-editing protein is A20. Encoded by the tumour necrosis factor alpha-induced protein 3 (TNFAIP3), A20 binds directly to the RANK receptor complex through zinc finger domains ZnF4 and ZnF7. A20 restricts NF-κB activation, limits osteoclast differentiation, and maintains bone homeostasis in RA [77].

Salubrinal is a synthetic compound that exhibits dual anti-inflammatory and anti-osteolytic effects in RA models. In CIA mice, salubrinal alleviated joint swelling, suppressed synovial inflammation, and inhibited osteoclastogenesis. This is achieved by promoting the ubiquitination and proteasomal degradation of the NF-κB subunit p65. This targeted degradation leads to reduced nuclear translocation of p65, thereby attenuating the transcription of downstream proinflammatory genes such as IL-1β and TNF-α, as well as limiting osteoclast formation. The ability of Salubrinal to modulate NF-κB activity through enhanced ubiquitination supports its promise as a disease-modifying agent in RA [78].

Taken together, these therapeutic agents exemplify the translational relevance of targeting the ubiquitin–proteasome system in RA. Whether by enhancing beneficial ubiquitination (e.g., auranofin, menthone, salubrinal) or suppressing pathological deubiquitination (e.g., USP5), these interventions provide different directions for managing inflammation, synovial aggression, and bone destruction in RA.

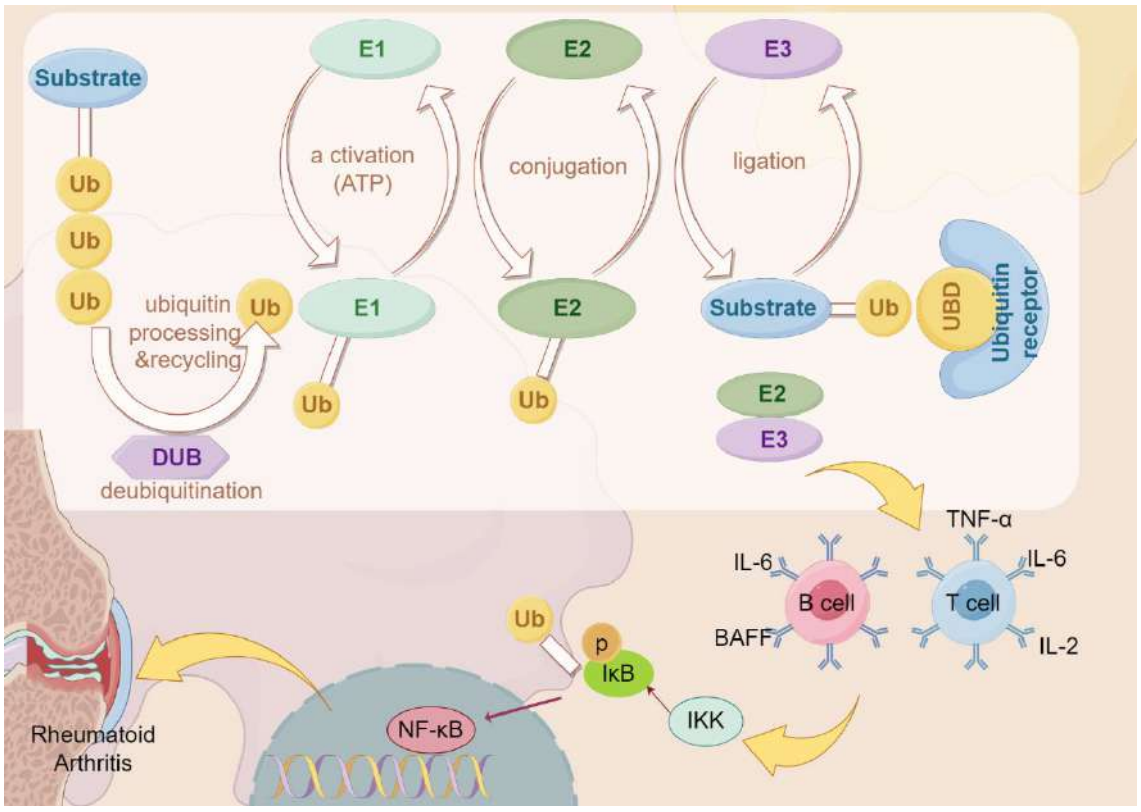

Figure 3: Ubiquitination Promotes RA Progression

This figure illustrates the ubiquitination cascade and its pathological contribution to RA. At the top, the canonical ubiquitination process is shown. Ub is activated by E1 enzymes in an ATP-dependent manner, transferred to E2 conjugating enzymes, and subsequently ligated to substrate proteins by E3 ligases. These ubiquitin-tagged substrates are recognized by ubiquitin receptors via their UBD, targeting them for degradation or signalling. DUBs can remove ubiquitin chains, adding another layer of regulation (Image prepared using Figdraw; ID: auytud02e2).

In the context of RA (bottom panel), ubiquitination facilitates the degradation of IκB, an inhibitor of NF-κB. This allows NF-κB translocation into the nucleus, where it promotes transcription of inflammatory genes. Consequently, proinflammatory cytokines such as TNF-α, IL-6, IL-2, and BAFF are released from immune cells like B and T lymphocytes. These mediators amplify inflammation, sustain immune activation, and drive joint destruction characteristic of RA.

Ub: Ubiquitination, IκB: Inhibitor of κB, IKK: IκB Kinase, BAFF: B-cell Activating Factor, UBD: Ubiquitin-Binding Domains, DUBs: Deubiquitinating Enzymes

Table 2: Roles of Selected Proteins, Small Molecules, and Phytochemicals in the Ubiquitination Pathway and Their Implication in RA.

Agent	Mechanism of Involvement in Ubiquitination	Role in RA	Reference
STUB1 (E3 Ligase)	Mediates non-degradative ubiquitination of AHR, disrupting Th17/Treg homeostasis	Contributes to immune dysregulation and RA progression	[65]
TRIM32 (E3 Ligase)	Promotes K63-linked polyubiquitination of TRAF2	Increases cytokine production in synovial cells, aggravates RA pathology	[67]
Mid1 (E3 Ligase)	Overexpressed in RA synovial tissues; Modulates Treg stability and synovial inflammation	Promotes pathological changes and accelerates disease progression	[71]
ZNRF3 (E3 Ligase)	Regulates NF-κB and Wnt pathways	Reduces pro-inflammatory mediator expression; Protects against joint damage	[76]
A20 (Ubiquitin-editing enzyme)	Binds RANK complex via ZnF4/ZnF7; regulates ubiquitin chain editing	Inhibits NF-κB activation; Prevents osteoclastogenesis and bone erosion	[77]
USP2 (DUB)	Deubiquitinates TRAF2	Promotes FLS proliferation and proinflammatory signalling	[66]
USP5 (DUB)	Removes ubiquitin from NF-κB regulatory proteins	Upregulates TNF-α, IL-6, IL-1β via NF-κB; correlates with RA severity	[74]
Auranofin	Enhances ubiquitination and proteasomal degradation of misfolded ER proteins via UBA1 activation	Alleviates ER stress, reduces inflammation	[69]
Salubrinal	Promotes K48-linked ubiquitination and degradation of NF-κB p65	Inhibits RANKL-induced osteoclastogenesis, alleviates arthritis symptoms	[78]
D3R (Dopamine Receptor)	Facilitates ubiquitination and lysosomal degradation of TLR4	Suppresses TLR4/NF-κB signalling, reduces proinflammatory cytokine production	[70]
IAA (β-Indole-3-acetic acid)	Reduces Foxp3 ubiquitination via AhR–TAZ–Tip60 complex	Enhances Treg differentiation, alleviates autoimmune inflammation	[72]
Menthone	Promotes K48-linked polyubiquitination of Tyk2, leading to proteasomal degradation	Inhibits IFN-I signalling, reduces synovial inflammation and joint swelling	[73]

7. Mechanisms of Ubiquitination and the MAPK Signalling Pathway in RA

Having established the individual roles of the MAPK signalling cascade and the ubiquitin-proteasome system in RA, increasing evidence suggests that these two pathways are functionally interconnected. Ubiquitination exerts regulatory control over multiple components within the MAPK pathway, thereby influencing inflammatory responses and synovial pathology characteristic of RA. This crosstalk highlights an additional layer of complexity in disease progression and offers potential combinatorial therapeutic targets.

Ubiquitination regulates MAPK signalling by modifying the stability, activation, and spatial organisation of upstream kinases and adaptor proteins. For example, MAP3K1 (MEKK1), a key upstream kinase in the MAPK cascade, undergoes distinct forms of ubiquitination. K48-linked ubiquitination targets MEKK1 for proteasomal degradation, suppressing downstream ERK and JNK activation, while K63-linked chains promote the assembly of signalling complexes that sustain MAPK activation. These opposing effects are dynamically reversed by DUBs such as CYLD and USP18, depending on the inflammatory context [79].

Additionally, specific E3 ligases such as Itchy E3 ubiquitin protein ligase (ITCH) have been implicated in modulating MAPK-related immune escape mechanisms. ITCH mediates the K48-linked ubiquitination and degradation of programmed death-ligand 1 (PD-L1). This effect is specifically enhanced during MAPK inhibitor therapy. While this mechanism is well-characterised in cancer immunotherapy, it reflects broader principles of immune modulation that may be relevant in controlling the immune escape of RA-FLSs and restoring T cell-mediated regulation [80].

The influence of ubiquitination also extends to scaffold proteins. RNF167 mediates the polyubiquitination of Toll-interacting protein (Tollip), leading to suppression of TNF-α-triggered activation of p38 MAPK, JNK, and NF-κB. This demonstrates how ubiquitination-dependent degradation of scaffold proteins can attenuate inflammatory MAPK signalling [81].

Receptor-interacting protein kinase 1 (RIPK1) is another key mediator of MAPK signalling whose function is tightly controlled by ubiquitination. IAP family E3 ligases (e.g., cIAP1/2) conjugate K63-linked polyubiquitin chains to RIPK1. This facilitates the recruitment of TAK1 and MKK complexes, thus activating JNK and p38 MAPK pathways. Inhibition of IAPs disrupts this activation axis, underscoring the regulatory role of RIPK1 ubiquitination in MAPK signalling output [82].

In RA, the interplay between ubiquitination and MAPK signalling is further supported by evidence from synovial tissue. Notably, UBD is markedly upregulated in RA-FLSs and correlates with increased phosphorylation of p38 MAPK. UBD promotes FLS proliferation, cytokine secretion (e.g., IL-2 and TNF-α), and resistance to apoptosis. Although the precise mechanism remains unclear, these effects suggest that UBD acts as an activator of MAPK signalling through a ubiquitin-dependent mechanism [11].

Together, these findings underscore that ubiquitination modulates MAPK signalling in RA not only by directly modifying key kinases such as MEKK1, RIPK1, and p38, but also by regulating adaptor and scaffold proteins like Tollip and PD-L1. This intricate interplay contributes to chronic inflammation, synovial hyperplasia, and immune dysregulation observed in RA. Further investigation into specific ubiquitin chain types (e.g., K48 vs. K63), lysine residues involved, and their crosstalk with phosphorylation may uncover new therapeutic strategies aimed at jointly targeting both MAPK and ubiquitin-mediated mechanisms.

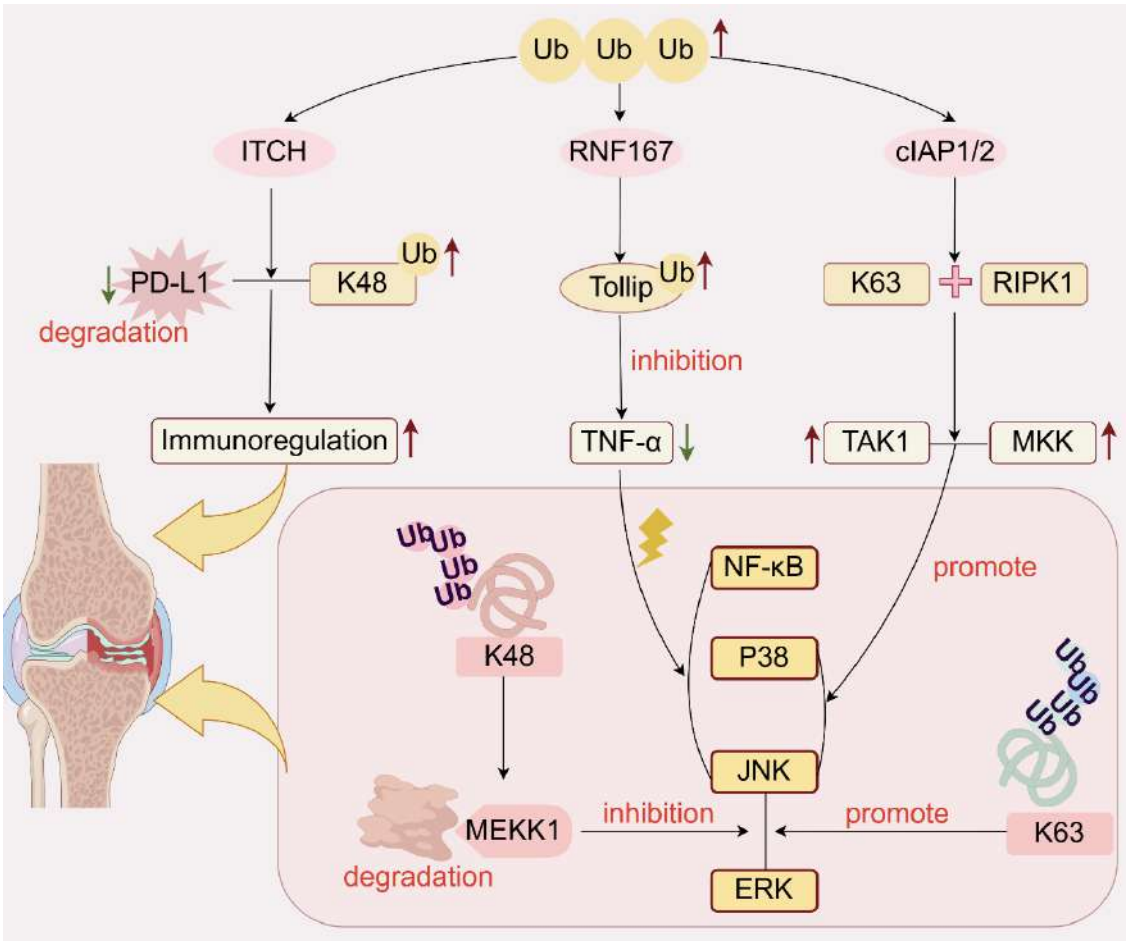

Figure 4: Mechanistic interplay between ubiquitination and MAPK signalling in rheumatoid arthritis

This schematic illustrates the crosstalk between ubiquitination and the MAPK signalling pathway in rheumatoid arthritis (RA). In RA fibroblast-like synoviocytes (RA-FLSs), the E3 ligase ITCH promotes K48-linked ubiquitination of PD-L1, leading to its proteasomal degradation and enhanced immunoregulation.

RNF167 mediates the ubiquitination of the scaffold protein Tollip, suppressing TNF-α-induced activation of MAPK and NF-κB signalling. cIAP1/2 catalyse K63-linked ubiquitination of RIPK1, facilitating TAK1 and MKK recruitment, thereby promoting JNK and p38 activation. MEKK1 is subjected to K48-linked ubiquitination, resulting in its degradation and inhibition of ERK signalling, while K63-linked ubiquitination promotes MAPK activation.

These ubiquitin-mediated modifications collectively modulate inflammatory signalling, immune escape, and synovial pathology characteristic of RA (Image prepared using Figdraw; ID: PWOPR79795).

8. Clinical Applications and Therapeutic Prospects

Therapeutic strategies targeting the MAPK signalling pathway have shown considerable promise in alleviating RA-related inflammation and joint destruction. For instance, MK2 inhibitors modulate chemokine profiles in synovial fluid, exerting anti-inflammatory effects by shifting monocyte recruitment patterns [83]. Natural compounds such as Tanshinone IIA, Paris saponin VII, and Lipoxin A4 have been reported to alleviate RA symptoms in animal models by suppressing MAPK pathway components, particularly JNK and p38. These resulted in reduced cytokine production and synovial hyperplasia [84-86]. Essentially, the findings highlight the feasibility of selectively targeting MAPK signalling elements for RA intervention.

Beyond MAPK signalling, compounds that regulate ubiquitination are emerging as promising therapeutic candidates. RES modulates SIRT1/Nrf2 signalling, as well as reduces oxidative stress and FLS proliferation via ubiquitination-related pathways. Similarly, sesamol enhances p53 stability by inhibiting its ubiquitination, thereby suppressing aberrant synovial activity [87, 88]. In addition, SYVN1 (an endoplasmic reticulum-resident E3 ligase) along with its associated non-coding RNAs, have been proposed as potential biomarkers for disease progression and treatment responsiveness in RA [89].

Dual-target approaches that modulate both MAPK signalling and ubiquitination offer a more comprehensive strategy for disease control. For instance, UBD promotes RA-FLS proliferation and inflammatory cytokine secretion via p38 activation, and its effects can be partially reversed by p38 MAPK inhibitors [11]. Furthermore, deubiquitinating enzymes regulate key bone-related signalling pathways, including bone morphogenetic protein/transforming growth factor-beta (BMP/TGF-β), Wnt/β-catenin, Epidermal growth factor receptor (EGFR)-MAPK, underscoring their relevance in inflammatory bone remodelling and joint preservation [90].

In summary, the integration of MAPK pathway modulation and ubiquitination control represents a promising therapeutic avenue in RA. These combined approaches may offer synergistic efficacy in reducing inflammation, preserving joint integrity, and improving long-term patient outcomes. Nevertheless, further clinical validation is required to translate these insights into effective combination therapies.

9. Summary and Outlook

RA is a multifaceted autoimmune disease driven by the interplay of cytokines, immune cells, and intracellular signalling pathways within the synovial microenvironment. Growing evidence highlights the central roles of MAPK signalling and ubiquitin-proteasome system in orchestrating inflammation, immune dysregulation, and joint damage. Specifically, MAPK signalling influences cytokine expression and FLS proliferation, while ubiquitination modulates the activity, stability, and localisation of MAPK components and related adaptor proteins.

Despite growing evidence supporting the pathological relevance of these pathways, key mechanistic questions remain unresolved. In particular, the context-dependent functions of specific ubiquitin-related enzymes (e.g., E3 ligases and deubiquitinases) on MAPK subunits (e.g., ERK, JNK, and p38) within the inflammatory microenvironment of RA require further elucidation. The functional consequences of different ubiquitin chain types (e.g., K48- vs. K63-linked) on MAPK activation, protein stability, and nuclear translocation in synovial fibroblasts or immune cells remains largely undefined. Moreover, it is also worth noticing that most mechanistic insights have been derived from in vitro systems or animal models, while direct evidence from patient-derived tissues remains scarce, posing a challenge for clinical translation.

To advance the field, future research should leverage integrated multi-omics approaches and patient-derived models to uncover the dynamic regulatory networks between ubiquitination and MAPK signalling at the post-translational level.

Identifying pathway-specific ubiquitin modifiers or scaffold proteins that selectively modulate MAPK activity holds promise for the development of precise, low-toxicity therapeutics. Furthermore, elucidating how these regulatory networks contribute to treatment resistance may help explain disease heterogeneity and improve patient stratification in RA.

In conclusion, targeting the convergence of ubiquitination and MAPK signalling represents a promising frontier in RA therapy. Strategic combination approaches that disrupt key inflammatory nodes could usher in a new era of personalised, mechanism-based treatment strategies for patients with refractory or progressive RA.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

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DECLARATION OF AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The English language of the article was improved with ChatGPT. Upon generating draft language, the author reviewed, edited and revised the language to their own liking and takes ultimate responsibility for the content of this publication.

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CONFLICT OF INTEREST

The authors declare that none of them has any conflict of interest.

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