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Exploring mammalian target of rapamycin (mTOR) Gene Expression and Its Implications in Rheumatoid Arthritis Pathogenesis and Therapy

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Abstract: Background: Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder characterized by persistent synovial inflammation, progressive joint damage, and debilitating functional impairment. The pathophysiology of RA is driven by complex interactions between genetic, immunological, and environmental factors, leading to dysregulated immune responses and aberrant cellular signaling pathways. Among these, the mammalian target of rapamycin (mTOR) pathway has garnered significant attention due to its central role in cellular growth, metabolism, and immune regulation. mTOR signaling influences key processes such as T-cell activation, macrophage polarization, synovial fibroblast proliferation, and the production of pro-inflammatory cytokines, all of which contribute to RA disease progression. Dysregulation of mTOR gene expression has been implicated in promoting synovial hyperplasia, angiogenesis, and the perpetuation of chronic inflammation in RA. This review comprehensively evaluates the current evidence on mTOR gene expression in RA, exploring its molecular mechanisms, functional consequences, and therapeutic implications. We analyze findings from preclinical studies, in vitro experiments, and emerging clinical trials investigating the use of mTOR inhibitors, such as rapamycin and its analogs, as potential disease-modifying agents. A deeper understanding of mTOR signaling in RA pathogenesis may pave the way for novel targeted therapies, offering improved outcomes for patients with this debilitating condition.

Keywords: *mammalian target of rapamycin, Gene Expression, Rheumatoid Arthritis*

Introduction

Rheumatoid arthritis is a chronic systemic autoimmune disease that affects more women than men [1]. It primarily affects the synovial joints, leading to cartilage degradation, joint erosion, and inflammation (synovitis). Many patients experience diminished functional status and impairment because of this disease. Extra-articular manifestations, which can impact the majority of the body's organs and increase mortality and morbidity rates, is another way that RA can present itself [2].

It is characterized by swelling, pain, and synovial joint inflammation, most frequently metacarpophalangeal, proximal interphalangeal, and metatarsophalangeal, wrist and knee joints [3].

It is a systemic disease with morning stiffness, constitutional symptoms, and extraarticular multisystem manifestations like subcutaneous nodules, vasculitis, pulmonary, cardiovascular, neurologic, and hematologic involvement. Symmetric polyarthritis is the main symptom, affecting small joints [4].

Rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) (the latter of which is more specific for the illness) are the two related autoantibodies that help in the diagnosis of RA [5]. Tumor necrosis factor (TNF)-α and interleukin (IL)-6 are two cytokines that cause an environment that is pro-inflammatory in this condition [6], and furthermore by a reduction in regulatory T cells (Tregs) [7].

It is uncertain what causes rheumatoid arthritis. Obesity, smoking, and exposure to air pollution are risk factors. The risk of having RA is higher in women and the elderly. Pharmacological treatment can limit symptoms and the progression of the disease if the diagnosis is made in early stages. Rehabilitation, which includes the use of assistive equipment, can maintain normal functioning. Surgical treatments, such as joint replacement, may be able to manage discomfort, preserve physical function, and aid restore movement in cases of severe joint injury [8].

The prevalence of rheumatoid arthritis (RA) is estimated to be between 0.24 and 1% worldwide; however, regional variations exist. Due to variations in socioeconomic status, environment, and ethnicity, it is also anticipated that RA prevalence will vary throughout Africa and the Middle East. In both urban and rural populations, RA was more common in women than in males. The prevalence ratio of women to men varied from 1.3:1 to 12.5:1, indicating significant deviations from the worldwide average of 2:1. Socioeconomic variables, delayed rheumatologists visit, and low disease knowledge seem to be barriers to early diagnosis and intensive therapy [9]. Rheumatoid arthritis (RA) represented one of the main autoimmune diseases in Egypt. They mainly affect young patients below the age of 40 years, most of them females [10-14].

While RA can strike people in almost any age range, most people with the disease first develop between the ages of 40 and 70 [13]. The incidence of RA in persons under 35 years of age is only 0.3%.

Risk factors:

Rheumatoid arthritis (RA) is influenced by both environmental and genetic variables [15]. Twin studies have shown that genetic variation accounts for 50 to 60% of the risk on RA development [16].

Genetic risk factors:

In particularly, for ACPA-positive RA, the strongest genetic risk factor for the development of RA is the HLA-DRB1*01, *04, and *10 alleles [17]. The majority of HLA-DRB1 alleles linked to RA have the same amino acid sequence in the peptide-binding groove known as the shared epitope (SE) [18].

The theory that all predisposed HLA molecules with the SE sequence may present “arthritogenic” peptides, potentially causing a joint-specific autoimmune reaction, is based on the sequence's similarity. There has been speculated that peptides expressed by alleles carrying SE could be citrullinated due to their high correlation with ACPA-positive RA. At the peptide-SE interaction region, it has been demonstrated that an arginine can be converted to a citrulline [19]. Antigen-presenting cells (APCs) may have more HLA peptide complexes on their surface as a result of SE's strong affinity for citrullinated peptides, which could trigger a (joint-specific) T cell response [20].

Additionally, as SE alleles also operate as a ligand for cell surface calreticulin (CRT), an innate immune receptor found on most human cells, particularly on dendritic cells, various theories regarding the role of the SE in the development of RA have also been proposed. The SE-CRT interaction can start a signal transduction cascade that alters the phenotypic of dendritic cells, skewing T cell responses to the T helper 17 (Th17) fraction and reducing the production of regulatory T cells. This interaction is more effective when CRT is citrullinated [21, 22].

"While substantial research has explored the role of mTOR signaling in immune regulation and inflammation, limited studies have investigated the specific mechanisms of mTOR gene expression in synovial tissue and immune cells in Rheumatoid Arthritis (RA) patients. Moreover, the differential effects of mTOR inhibition on various immune cell subsets (e.g., T-cells, macrophages, fibroblast-like synoviocytes) remain underexplored, particularly concerning disease progression and therapeutic resistance. Further investigation into these molecular mechanisms may uncover novel therapeutic targets and improve precision in mTOR-based treatments for RA." This gap emphasizes: Lack of focus on mTOR gene expression specifically in RA tissues and immune cells. Mechanistic understanding of mTOR's impact on cell-specific pathways. Therapeutic resistance and heterogeneity in response to mTOR inhibitors.

Rapamycin (mTOR) in rheumatoid arthritis

Multiple single-nucleotide polymorphisms (SNP), in addition to the HLA region, are linked to rheumatoid arthritis [23]. The PTPN22 gene, the second most potent genetic risk factor for the development of RA [24]. Tyrosine phosphatase (PTPs), which is involved in T cell and B cell antigen receptor (TCR) signaling, is encoded by the PTPN22 gene. Therefore, the fact that this gene is linked to a number of autoimmune disorders [25].

Rapamycin (Figure 1) is a 31-membered macrocyclic natural product produced by several actinomycetes, including *Streptomyces rapamycinicus* (formerly *Streptomyces hygroscopicus* ATCC 29253), *Streptomyces iranensis*, and *Actinoplanes* sp. N902-109. It was first found in terrestrial bacteria collected on Easter Island in a screening of antifungal agents [31].

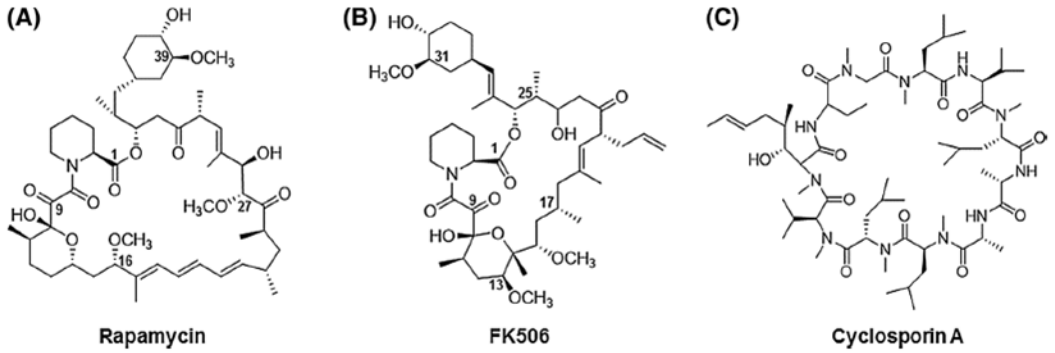

Figure 1: Structure of immunosuppressive agents, a rapamycin, b FK506, and c cyclosporin A [31].

Later, it was revealed that rapamycin possessed immunosuppressive, antitumor, neuroprotective/neuroregenerative, and lifespan extension activities. This broad range of biological activities inspired interest in its target and mode of action. Rapamycin inhibits mammalian target of rapamycin (mTOR) (Figures 2,3) by associating with its intracellular receptor FK506-binding protein 12 (FKBP12). The FKBP12-rapamycin complex interacts directly with the FKBP12-rapamycin-binding (FRB) domain of mTOR, which belongs to the phosphoinositide kinase-related kinase family. Once loaded onto the FRB domain, the FKBP12–rapamycin complex inhibits mTOR which associates with various proteins in mammals and is important in the life of the cell [32].

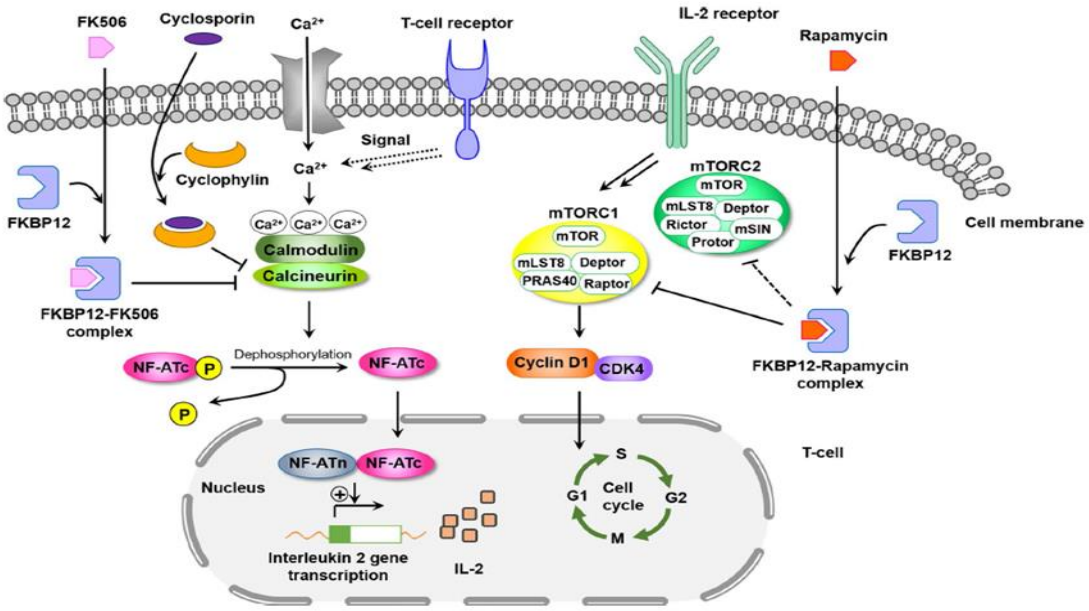

Figure 2: Mechanism of action of immunosuppressive agents such as rapamycin, FK506, and cyclosporin. Rapamycin inhibits mammalian target of rapamycin (mTOR) by binding to its intracellular receptor FK506-binding protein 12 (FKBP12) [32].

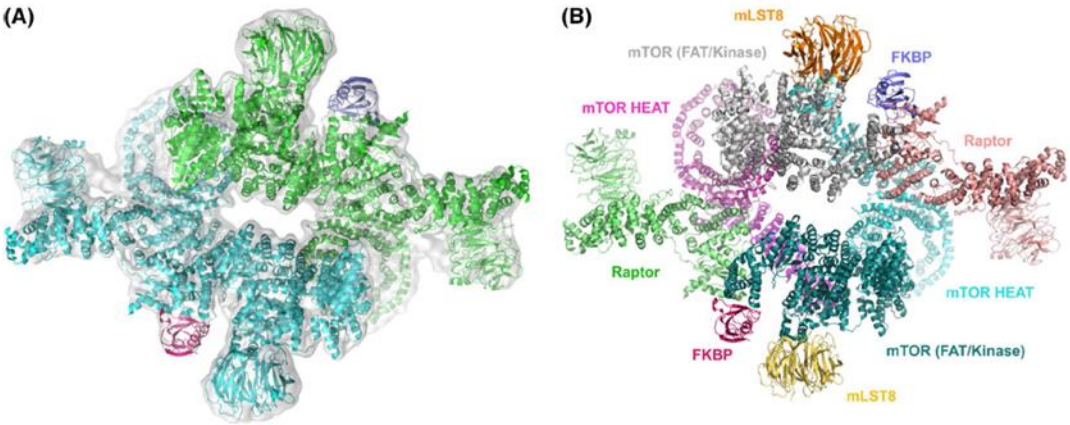

Figure 3: Cryo-EM structure of the mTORC1 core complex with FKBP-rapamycin. a mTORC1 shows a hollow dimer with rhomboidal shape. Monomer units are shown with green and cyan colors.

b Each component of mTORC1 is shown with different colors in the ribbon diagram [33].

At the joint inflammatory site, abnormal modulation of mTOR signaling results in continuous feedback between stromal cells and infiltrating immune cells, leading to persistent inflammation and tissue damage, driving the pathological tissue refactoring and eventually causing organ dysfunction. Activation of the mechanistic target of rapamycin (mTOR) affects the abundance and functioning of immune and stromal cells and may thus be an essential pathway in the pathogenesis of RA [34].

The regulators of the mTOR pathway have been the subject of several studies that, recently, expanded to the understanding of how mTOR expression itself is regulated. Furthermore, the investment in the development of biomarkers has been exponential and, in this scope, several authors are addressing the expression of mTOR in

different pathological conditions, such as Type 2 Diabetes Mellitus, Alzheimer's disease, rheumatoid arthritis, and in several types of cancers [35].

mTOR signalling:

Metabolic regulatory networks: Signalling pathways that control the proliferation, survival and differentiation of cells in the immune system also regulate the metabolic processes that provide the nutrients required to support these specialized lymphocyte functions. Although mTOR drives proinflammatory lineage specification in the T cell compartment, it might also have anti-inflammatory effects driven by shifting macrophage polarization from a proinflammatory M1 phenotype to an anti-inflammatory M2 phenotype. Nevertheless, M2 macrophages can also contribute to inflammation, for example by serving as hosts to cytomegalovirus (CMV), which might explain the potent anti-CMV effects of mTOR inhibitors after organ transplantation [35]. Outside the immune system, mTOR activation contributes to type I collagen production by dermal fibroblasts and to fibrosis in patients with SSc. In chondrocytes, genetic inactivation of mTOR prevented the development of OA induced by peroxisome proliferator-activated receptor γ (PPARγ) deficiency. Furthermore, mTOR blockade with N acetylcysteine (NAC), an amino acid precursor of glutathione, reduced cognitive attention deficit hyperactivity disorder symptoms in patients with SLE. Given all the evidence for a pathogenetic role for mTOR in rheumatic diseases and associated comorbidities, the metabolic pathways sensed and regulated by this signalling molecule are important to understand [36].

Stimulatory and inhibitory signal transducers: The mTOR pathway is largely controlled by upstream checkpoints at three levels: receptor tyrosine kinases and G-protein-coupled receptors, which detect growth factors; the PI3K–PDK1 (phosphoinositide-dependent kinase 1)–AKT (RAC-α serine/threonine-protein kinase) axis, which channels stimulatory signals towards mTORC1 activation; and the key negative regulators PTEN, AMPK (5ˈ AMP-activated protein kinase catalytic subunit α2), TSC1 and TSC2 (the latter two are also known as hamartin and tuberin, respectively) [37].

Cell-type-specific activation of mTOR: The mTOR pathway has critical roles in the development and function of the innate and adaptive arms of the immune system. An essential role of mTOR in T cell development was uncovered when mTORC1 was found to be required for the differentiation of TH1 and TH17 cells, whereas TH2 cell development depends on mTORC2. mTORC1 also inhibits the survival of CD8+ memory T cells, an effect that can be reversed by rapamycin treatment. Both mTORC1 and mTORC2 seem to interfere with the differentiation and function of CD4+CD25+FoxP3+ TREG cells, but mTORC1 might also support TREG-cell function by inhibiting the mTORC2 pathway [38].

Despite its complex role in TREG-cell function, the largely proinflammatory changes elicited by mTORC1 activation in the adaptive immune system are in apparent contrast with its anti-inflammatory effects on the innate immune system (for example, mTORC1 favours M2 over M1 polarization of macrophages). The cell-type-specific activation of mTORC1 also varies between rheumatic diseases, as mTORC1 is activated in T cells of patients with SLE, but not in those of patients with RA. These divergent effects of mTOR activation in T cells might be connected to alternative use of glucose between glycolysis and the PPP [39].

Upstream regulation of mTOR: Several ligands activate mTOR signaling, including growth factors, amino acids, antigens, and cytokines. The pathways of mTORC1 activation have been extensively researched; however, mTORC2 regulation is currently less understood. Compared to mTORC1, mTORC2 is an insensitive signaling factor [40].

The activity of mTORC1 is primarily mediated by phosphatidylinositol-3-OH kinase/RAC-α serine/threonineprotein kinase/tuberous sclerosis complex (PI3K/AKT/TSC) and liver kinase B1/the mitogen-activated protein kinase (LKB1/AMPK) axis. Growth factor receptor, cytokines, and Toll-like receptor (TLR) ligands regulate mTORC1 activity by the PI3K/AKT/TSC signaling pathway. They phosphorylate PI3K to activate downstream effector AKT, then restrain the TSC1/TSC2 complex. Ras homolog enriched in the brain (Rheb) primarily occurs in a GTP-bound activated state [41].

Inactivation of the TSC complex restrains the hydrolysis of Rheb-GTP, preventing it from conversion into Rheb-GDP. Finally, Rheb-GTP directly activates mTORC1. Through continuously improving understanding of the pathway, phosphatase and tensin homolog deleted on chromosome 10 (PTEN) was found to neutralize the activity of PI3K by dephosphorylating the downstream products of PI3K, which include phosphatidylinositol-3,4-bisphosphate (PIP2) and phosphatidylinositol-3,4,5-triphosphate (PIP3). The most remarkable characteristic of mTORC1 is its activating ability on the surface of the lysosomal membrane [42].

Growth factors activate Rheb, and amino acids recruit mTORC1, both of which are indispensable for the activation of mTORC1. AMPK regulates glucose metabolism and perceives changes in ATP levels. Energy stress activates LKB1-dependent AMPK, which phosphorylates downstream TSC2, then promotes the formation of the TSC1/TSC2 complex, and further inhibits the activity of mTORC1. In addition, AMPK directly phosphorylates raptor and suppresses mTORC1 signaling (figure 11A) [43].

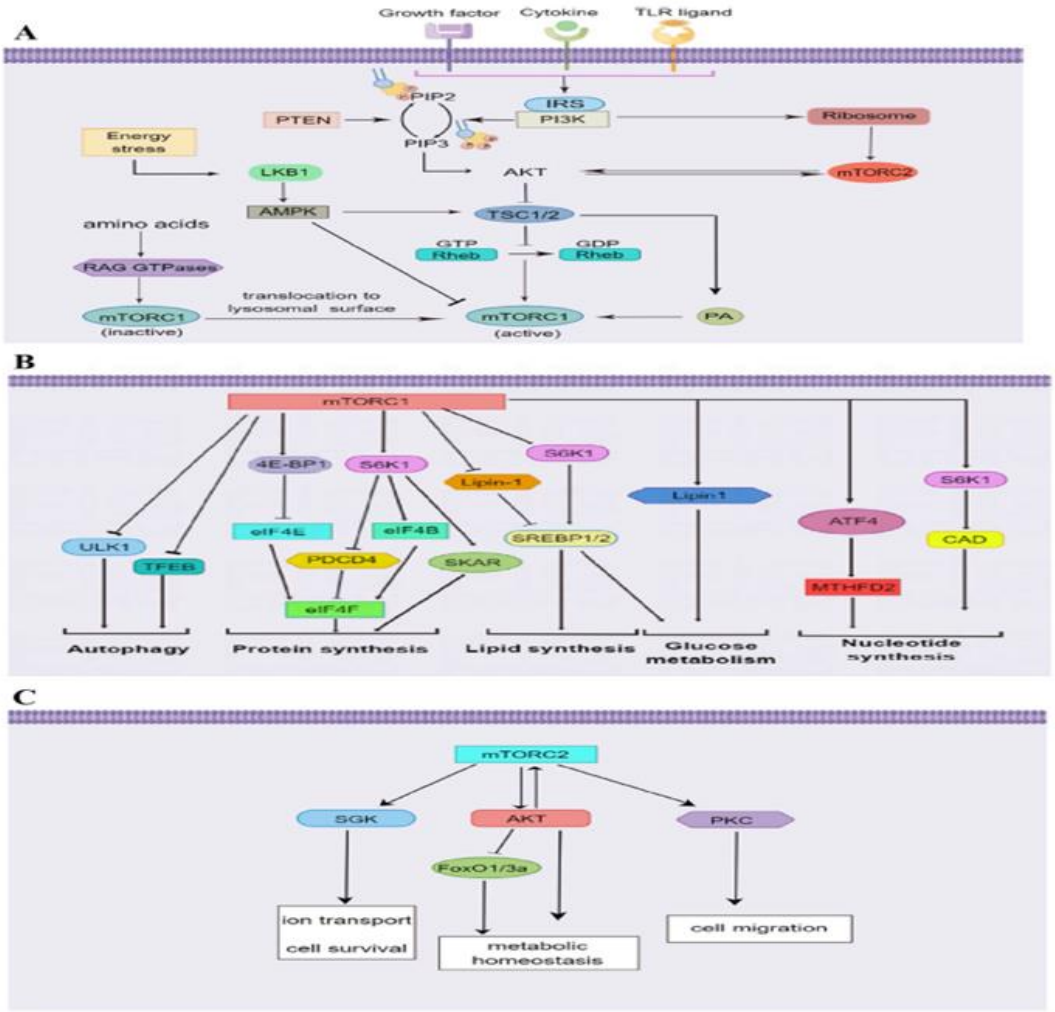

Figure (4) A The upstream pathway of mTORC1 and mTORC2. Growth factor, cytokines, or TLR signaling activates PI3K, which in turn activates AKT. The AMPK, LKB1, and IRS are also involved in regulating mTORC1 and mTORC2. TLR, Toll-like receptor; PI3K, phosphatidylinositol-3-OH kinase; AKT, RAC-α serine/threonine-protein kinase; TSC, tuberous sclerosis complex; Rheb, Ras homolog enriched in the brain; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PIP2, ephosphatidylinositol-3,4-bisphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; AMPK, the mitogen-activated protein kinase; LKB1, liver kinase B1, IRS, insulin receptor substrate. B The downstream pathway of mTORC1. mTORC1 activates S6K1 and eIF4E to promote protein synthesis and cell growth. mTORC1 also inhibits 4E-BP1 to initiate translation. Additionally,

mTORC1 influences PDCD4, SKAR, ATF4, MTHFD2, CAD, HIF1α, ULK1, and TFEB to regulate glucose metabolism, lipid synthesis, and autophagy. S6K1, S6 kinase 1; eIF4E, eukaryotic translation initiation factor 4E; eIF4B, eukaryotic translation initiation factor 4B; 4E-BP1, the eIF4E binding protein 1; PDCD4, programmed cell death protein 4; SKAR, S6K1 Aly/REF-like target; ATF4, activating transcription factor 4; MTHFD2, methylenetetrahydrofolate dehydrogenase 2; CAD, carbamoyl-phosphate synthetase; HIF1α, hypoxia-inducible factor-1α; ULK1, unc-51-like kinase1; TFEB, transcription factor EB. C The downstream pathway of mTORC2. mTORC2 activates SGK1 and PKCα, which play roles in cell survival and cytoskeletal organization. SGK1, serum and glucocorticoid inducible kinase 1; PKCα, protein kinase C-α

Metabolic regulatory networks: Signalling pathways that control the proliferation, survival and differentiation of cells in the immune system also regulate the metabolic processes that provide the nutrients required to support these specialized lymphocyte functions. Although mTOR drives proinflammatory lineage specification in the T cell compartment, it might also have anti-inflammatory effects driven by shifting macrophage polarization from a proinflammatory M1 phenotype to an anti-inflammatory M2 phenotype. Nevertheless, M2 macrophages can also contribute to inflammation, for example by serving as hosts to cytomegalovirus (CMV), which might explain the potent anti-CMV effects of mTOR inhibitors after organ transplantation [35]. Outside the immune system, mTOR activation contributes to type I collagen production by dermal fibroblasts and to fibrosis in patients with SSc. In chondrocytes, genetic inactivation of mTOR prevented the development of OA induced by peroxisome proliferator-activated receptor γ (PPARγ) deficiency. Furthermore, mTOR blockade with N acetylcysteine (NAC), an amino acid precursor of glutathione, reduced cognitive attention deficit hyperactivity disorder symptoms in patients with SLE. Given all the evidence for a pathogenetic role for mTOR in rheumatic diseases and associated comorbidities, the metabolic pathways sensed and regulated by this signalling molecule are important to understand [36].

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The activity of mTORC2 is mainly regulated through the PI3K/AKT axis. In detail, PI3K induces PtdIns(3,4,5)P(3) to combine with the pleckstrin homology domain of msin1, blocking inhibition of the mTOR kinase domain by msin1, thereby activating mTORC2. PI3K also promotes the combination of the ribosome and mTORC2, and the ribosome is necessary for inducing mTORC2 kinase activity. Of note, partial activation of Akt boosts the activation of mTORC2, which phosphorylates and ultimately activates Akt, resulting in a positive feedback loop [44].

Downstream targets of mTOR: mTORC1 maintains metabolic homeostasis by regulating biological synthesis and catabolic processes. Activated mTORC1 phosphorylates its downstream targets S6 kinase 1 (S6K1) and the eIF4E binding protein 1 (4E-BP1) to regulate protein synthesis [45]. Phosphorylated 4E-BP1 releases cap-binding protein eukaryotic translation initiation factor 4E (eIF4E), which counteracts inhibition of protein synthesis by enabling eIF4E to form the eIF4F complex and to participate in cap-dependent translation [46]. S6K1 regulates eukaryotic translation initiation factor 4B (eIF4B), programmed cell death protein 4 (PDCD4),

and S6K1 Aly/REF-like target (SKAR) to participate in protein synthesis. eIF4B is a positive regulator of eIF4F complex, and PDCD4 is a negative regulator of eIF4A. The mTORC1 phosphorylates lipin 1 to increase the activity of SREBP1 or activates SREBP1 through an S6K1-dependent mechanism, thus participating in lipid synthesis [47].

In addition, mTORC1 promotes nucleotide synthesis by stimulating the mTHF cycle, which increases ATF4 levels to upregulate the expression of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2). Moreover, S6K1 phosphorylates carbamoyl-phosphate synthetase (CAD) to activate the de novo pyrimidine synthesis pathway. Furthermore, mTORC1 increases the expression of transcription factor HIF1α to promote glucose metabolism. SREBP1 is also involved in the regulation of the pentose phosphate pathway. Taken together, mTORC1 regulates various metabolic pathways to coordinate anabolism (figure 11B) [48].

mTORC1 negatively regulates catabolic processes such as autophagy to promote cell growth. Under nutrient sufficiency, activated mTORC1 phosphorylates and suppresses unc-51-like kinase1 (ULK1). Transcription factor EB (TFEB) regulates the expression of autophagy and lysosomal genes, which is also phosphorylated and inhibited by mTORC1 to regulate autophagy indirectly [49].

mTOR regulates dendritic cell (DC) differentiation, maturation, and function:

DCs are efficient antigen-presenting cells that initiate the initial immune response. During infection or under stressful conditions, mature DCs present antigens to naive T cells, resulting in the differentiation of effector CD4+ T cells, production of B cell antibodies, and activation of macrophages. DCs also contribute significantly to maintaining immune homeostasis and tolerance. DCs secrete chemokines to attract macrophages, neutrophils, and T cells to the synovium during RA, which potentiates subsequent immune responses [46].

The specific mechanisms of various pro-inflammatory immune cells are described in detail above. DCs are directly or indirectly involved in the development of RA, and interactions between DCs and Tregs also interfere with RA development. Tregs express cytotoxic T-lymphocyte-associated protein-4, lymphocyte-activating gene-3, and neuropilin-1 to suppress DC functions [47].

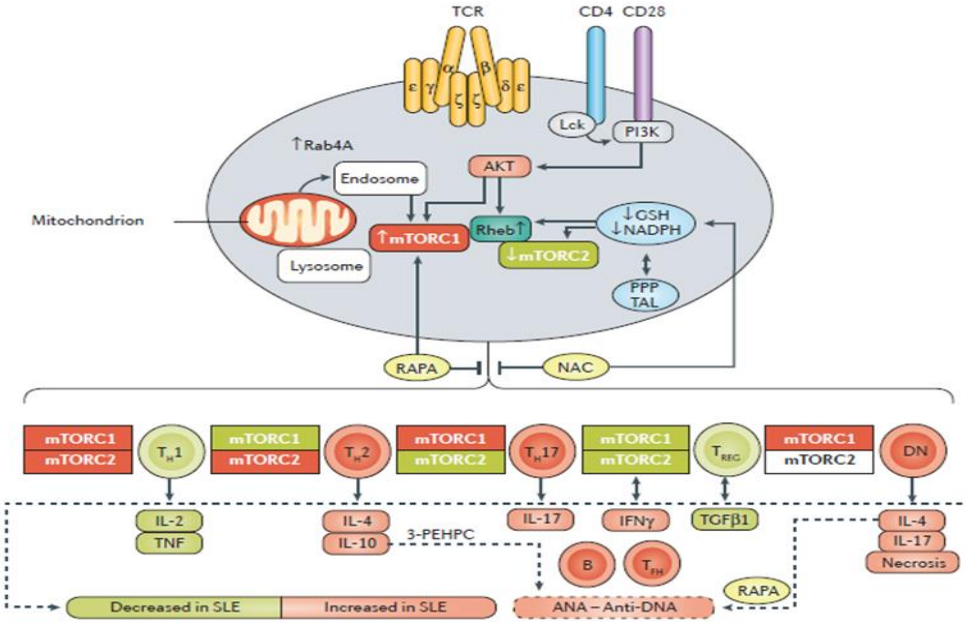

Figure 5: mTOR-mediated lineage specification in T cells [47].

The translocation of mTOR to the lysosomal mem¬brane occurs via endosome traffic that is regulated by Rab7, Rab5 and Rab4A. Each of these GTPases also regulates autophagy. Rab4A forms a positive feedback loop with mTORC1 and negative feedback loop with mTORC2, and binds to the p85 regulatory subunit of phosphatidylinositide 3 kinase (PI3K), altering its ability to control endocytic recycling. Notably, Rab4A and

Rab5 are overexpressed in T cells of patients with SLEand in mouse models of this disease; thus, these small GTPases are possible reg¬ulators of abnormal T cell signal transduction in SLE. Furthermore, overexpression of Rab4A, but not Rab5, also precedes the production of antinuclear antibodies (ANA) and SLE onset. Importantly, pharmacological inhibition of Rab GTPases by 3-(3 pyridyl)-2 hydroxy-2 phosphonopropanoic acid (3 PEHPC) prevents T cell dysfunction, ANA production, and nephritis in lupus prone mice

The suppressive effects of mTOR inhibition on DCs have been thoroughly investigated. Inhibition of mTOR reduces the expression of antigen uptake receptors and costimulatory molecules in DCs and restrains receptor-mediated phagocytosis. Furthermore, inactivation of mTOR was suggested to inhibit CD86 expression induced by TLR ligands (such as LPS) or CD40-specific during DCs differentiation [48].

mTOR inhibitors attenuate the HIF-1α pathway to suppress hypoxia-induced inflammation and affect the differentiation of immature DCs. mTOR is induced by Flt3 ligand (Flt3L) and is necessary for the Flt3L-driven development of DCs, and mTOR inhibits IL-1β production to impede DC maturation and the ability to stimulate effector T cell responses. In contrast, DCs under mTOR inactivation conditions favor differentiation of FOXP3+ Tregs [49]. In conclusion, mTOR exerts multiple effects on DC differentiation and functioning, which interferes with antigen uptake and presentation, and it affects cytokine production and chemokine receptor expression to modulate immune responses [50].

mTOR regulates the proliferation of RA FLSs:

Abnormal proliferation, as well as high invasion and migration of FLSs in joints, are the main characteristics of RA. Inflammation of RA-affected joints commences with FLS proliferation and invasion of immune cells into the synovium. FLSs then elicit overproduction of chemokines, cytokines, and matrix degradation molecules, thereby promoting immune cell infiltration and cartilage degradation. Additionally, activated FLSs migrate to the synovial sub-lining layer to promote angiogenesis and exacerbate synovial hyperplasia and bone destruction. In RA synovial cells, the mTOR signaling pathway is abnormally activated to participate in the regulation of RA FLS invasion [51].

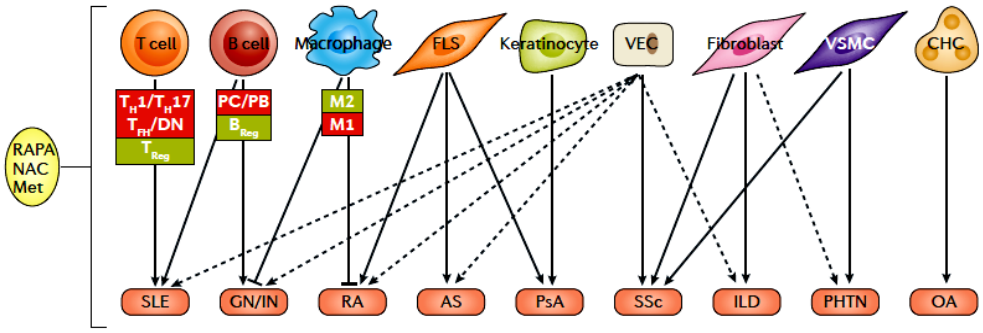

Figure (5): Cell type-specific mTOR pathway activation in rheumatic diseases [51].

Additional evidence of the benefits of mTORC1 inhibition in RA includes the ability of IL-17 to induce mTORC1-dependent proliferation of RA FLS, the increase in mTORC1 activity in osteoclasts from patients with RA and in arthritic transgenic mice, and the downregulation of extracellular matrix digestive enzymes and induction of apoptosis in osteoclasts elicited by mTOR inhibition. Taken together, these observations suggest a therapeutic benefit from mTOR blockade in RA that might involve the intra-articular cells that mediate erosive joint destruction

Aberrant activation of PI3K/Akt/mTOR leads to high expression of anti-apoptosis genes, reduced autophagy, and continuous proliferation of FLSs. Inactivating mTOR prevents FLSs from recombining the actin cytoskeleton and eliciting bone destruction [52].

mTOR regulates differentiation and formation of OCs:

OCs, differentiated giant multinucleated cells derived from the monocyte/macrophage lineage, decompose the bone matrix by producing various enzymes and acids. OCs are hyperfunctioning during RA, resulting in bone destruction. mTOR controls the autophagy pathway to participate in OC differentiation and formation [53].
Inhibition of autophagy blocks the differentiation of RA mouse macrophages into OCs, decreases bone erosion, and reduces OC abundances. Conditional deletion of mTOR (raptor) in OCs results in decreased OC differentiation and activity. The increased expression of structurally active S6K1 rescues damage of OCs differentiation under Raptor-deficiency. The AMPK/mTOR/p70S6K signaling pathway induces autophagy to inhibit OC differentiation, thus regulating bone mineral density and improving bone mass [54].
Furthermore, inhibition of the AMPK/mTOR/ULK1 pathway reduces autophagy in OCs exposed to high levels of glucose, and activation of the PI3K/AKT/mTOR axis suppresses autophagy of OCs treated with hydrogen sulfide. Additionally, pharmacological inhibition of mTOR induces OC apoptosis and inhibits OC activity and differentiation. In conclusion, elucidating the mTOR pathway provides essential insights into the molecular mechanism of regulating OCs [55].

mTOR-targeted therapy:

mTOR regulates many cellular processes and participates in the development of RA. Blocking this pathway through mTOR inhibitors is beneficial for RA treatment through changes in the immune and metabolic environment [50].

Rapamycin:

Rapamycin is a potent mTOR inhibitor that controls antigen-induced T-cell expansion, antibody production, and cellular proliferation. This compound was originally approved by regulatory authorities in 1999, with the purpose to prevent kidney allograft rejection. Further research revealed that mTOR is generally activated in neoplasms and controls cancer cell metabolism by regulating key metabolic enzymes; thus, it has been targeted for cancer treatment. Moreover, rapamycin inhibits the proliferation of endothelial cells and prevents the deterioration of disease by intra-arterial drug-eluting stents [56].
In short, rapamycin has been successfully used for numerous medical applications, such as anti-inflammation, anti-immune rejection, anti-tumor, and anti-endothelial proliferation treatments, and it plays a key role in the treatment of many diseases.
Rapamycin significantly inhibits the activity of arthritis and improves immune function in RA mice. Rapamycin was also successfully used in clinical trials for RA treatment [57]. [58] found that patients receiving rapamycin therapy achieved profound clinical improvement through increased circulating Tregs during RA. In addition, rapamycin reduces the secretion of inflammatory cytokines such as IL-6, TNF, and IL-1β, relieving the symptoms of RA.
Moreover, rapamycin also decreases the necessity for conventional disease-modifying antirheumatic drugs in controlling RA activities. Taken together, rapamycin induces autoimmune tolerance to reduce joint inflammation and is expected to be a new option for treating RA [58].

Rapalogues:

Semi-synthetic rapamycin analogs are collectively referred to as rapalogues, such as everolimus (also known as RAD001), temsirolimus (CCI779), and ridaforolimus. Compared with rapamycin, rapalogues are designed for higher water solubility and oral administration. Everolimus inhibits the proliferation of synovial cells and the activity of OCs, which affects bone erosion during RA. Everolimus has the advantages of a simple administration route (oral), low cost, and low risk of infection [59].
[60] investigated the safety and efficacy of everolimus and reported that everolimus plus methotrexate showed better clinical efficacy and reduced adverse reactions. Temsirolimus neutralizes the stimulation of LAT1 by IL-17 and reduces leucine uptake and fibroblast migration to prevent further erosion of the cartilage and bone.

Second-generation mTOR inhibitors:

Dual PI3K and mTOR inhibitor NVP-BEZ235 [61] decreases mTOR and Akt phosphorylation to accelerate the apoptosis of bone cells. Furthermore, the dual effects of BEZ235 on PI3K/Akt and mTOR signaling pathways

inhibit the activation of fibroblasts and eliminate the defect of rapamycin in p-Akt feedback activation of Ser473 after treatment with TGF-β [61].

N-acetylcysteine (NAC):

NAC is an antioxidant and anti-inflammatory agent whose functions are to promote glutathione biosynthesis and scavenge free radicals. Glutathione modulates T-cell differentiation by regulating mitochondrial transmembrane potential (Δψm) and mTOR [62].

During RA, oxidative stress stimulates OC formation and activation to promote bone resorption. NAC reduces IL-17-induced activation of the mTOR/JNK/NF-κB (nuclear factor κB) pathway to regulate the expression of RANKL in synovial fibroblasts and osteoblasts, preventing inflammation and bone destruction during RA. NAC supplementation improves clinical indicators of RA [63].

Additionally, continued exposure of T cells at sites of inflammation elicits high levels of reactive oxygen species (ROS), which results in decreased intracellular levels of GSH, dysregulation of redox balance, impaired signal transduction of the TCR/CD3 complex, and ultimately to synovial T cell hyporesponsiveness during RA [64].

Accumulation of ROS induces oxidative damage and chondrocyte senescence, thereby accelerating the degeneration of the cartilage matrix, and antioxidant NAC reverses this process. NAC effectively scavenges free-oxygen radicals, decelerates the process of cartilage degradation, reduces synovitis, and relieves pain [65].

Furthermore, NAC prevented chondrocyte apoptosis and cartilage destruction in an experimentally induced rat model of RA. However, long-term oral NAC administration is associated with a higher risk of RA [66].

Metformin (Met):

Met is the cornerstone of diabetes treatment, and during treatment of rheumatic diseases, it activates AMPK and inhibits mTORC1. Met-mediated AMPK activation and inhibition of mTOR activity regulate autophagy flux, inhibit NF-κB signal transduction and production of inflammatory cytokines, and reduce inflammation during experimentally induced arthritis. Met effectively inhibits the proliferation of RA-FLS by inducing G2/M cell cycle phase arrest, thus upregulating and downregulating phosphorylation of p70S6K and 4E-BP1 [67].

Furthermore, Met exerts an immunomodulatory effect on collagen-induced arthritis by inhibiting Th17 cell differentiation and upregulating Treg differentiation. Met inhibits differentiation of B cells into plasma cells and formation of spontaneous germinal centers through the AMPK/mTOR/STAT3 signal pathway and thus reduces autoantibodies production and inflammation [68]. [69] found that long-term use of Met is related to a reduced risk of developing RA.

Statins:

Statins are lipid-lowering drugs that prevent cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase, commonly used in treating hypercholesterolemia and cardiovascular-related diseases. Statins are also used as immunomodulators to block the adhesion of antigen-presenting cells to T cells, thus preventing the proliferation and function of T cells. Statins influence the activation of GTPases, such as Rho-GTPases, thereby regulating the transduction of PI3K/Akt/mTOR and ERK signaling pathways. The effects of statins on Tregs in RA patients in vivo and in vitro confirm that Tregs participate in the immunomodulatory impact of statins on RA [70].

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