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Review

Rho GTPase signaling in rheumatic diseases

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SUMMARY

Rho guanosine triphosphatase (GTPases), as molecular switches, have been identified to be dysregulated and involved in the pathogenesis of various rheumatic diseases, mainly including rheumatoid arthritis, osteoarthritis, systemic sclerosis, and systemic lupus erythematosus. Downstream pathways involving multiple types of cells, such as fibroblasts, chondrocytes, synoviocytes, and immunocytes are mediated by activated Rho GTPases to promote pathogenesis. Targeted therapy via inhibitors of Rho GTPases has been implicated in the treatment of rheumatic diseases, demonstrating promising effects. In this review, the effects of Rho GTPases in the pathogenesis of rheumatic diseases are summarized, and the Rho GTPase-mediated pathways are elucidated. Therapeutic strategies using Rho GTPase inhibitors in rheumatic diseases are also discussed to provide insights for further exploration of targeted therapy in preclinical studies and clinical practice. Future directions on studies of Rho GTPases in rheumatic diseases based on current understandings are provided.

INTRODUCTION

Rheumatic diseases are a set of inflammatory disorders predominantly affecting connective tissues and joints. Despite research advances, the pathogenesis of rheumatic diseases remains largely unknown, which hampers the progress in therapeutics. Rho guanosine triphosphatases (GTPases) are a family of signaling proteins containing 8 subfamilies, among which Cdc42, Rac and Rho are the best-characterized ones in various diseases including rheumatic disorders (Haga and Ridley, 2016).

Rho GTPases are essential molecular switches within cells (Woldu et al., 2018). The cycling of Rho GTPases between the inactive, guanosine diphosphate (GDP)-bound state, and the active, guanosine triphosphate (GTP)-bound state is responsible for their control of downstream signaling pathways (Woldu et al., 2018). Regulators including guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs) are involved in controlling this process. GEFs activate Rho GTPases by GTP loading, while GAPs and GDIs deactivate them by hydrolysis and GDP-bound state stabilization, respectively (Lawson and Ridley, 2018).

As molecular switches, Rho GTPases control a vast number of downstream pathways, and have well-recognized roles in mediating various cellular processes, such as actin cytoskeleton reorganization and immune response (Bros et al., 2019). In recent years, emerging studies have demonstrated the deregulation and involvement of Rho GTPases in rheumatic diseases.

In this review, we summarize the roles of Rho GTPases in the development and progression of rheumatic diseases, with a focus on rheumatoid arthritis, osteoarthritis, and systemic sclerosis. We also elucidate Rho GTPase-mediated pathways in rheumatic diseases. Finally, we discuss therapeutic strategies using inhibitors targeting Rho GTPases.

RHO GTPASES IN RHEUMATIC DISEASES

Rheumatoid arthritis

Pannus and fibroblast-like synoviocytes

Rho GTPases are involved in the pathogenesis of rheumatoid arthritis (RA) (Figure 1). Hypertrophied synovial tissues, namely, the pannus, contain macrophage-like synoviocytes (type A synoviocytes) and

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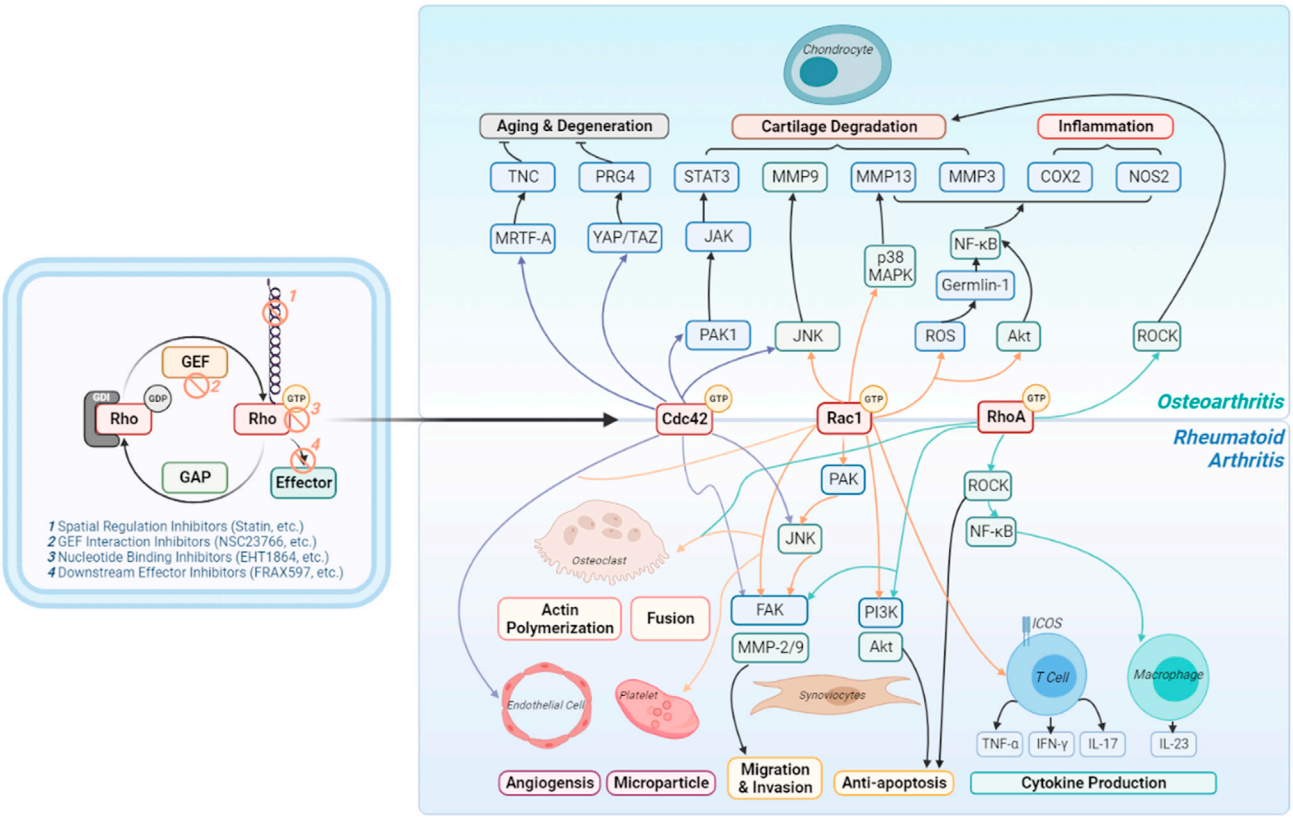

Figure 1. Role of Rho GTPases in rheumatoid arthritis and osteoarthritis

Rho GTPases in the active (GTP-bound) states activate various downstream pathways involved in rheumatoid arthritis and osteoarthritis. For osteoarthritis, Cdc42 increases the expression of TNC and PRG4 in chondrocytes through MRTF-A and YAP/TAZ, which are chondroprotective cartilage. In contrast, Cdc42 activates PAK to induce JAK/STAT3 activation and articular degeneration. Cdc42 and Rac1 activate JNK and further induce MMP-9 expression, which is responsible for collagen degradation. MMP3/13 are also regulated by Rac1 by p38 MAPK and Akt/NF-κB signaling. RhoA/ROCK activation also induces cartilage degradation. COX2 and NOS2 activated by Rac1 lead to inflammation. For rheumatoid arthritis, both Cdc42 and Rac1 enhance angiogenesis and platelet microparticle formation. Cdc42, Rac1, and RhoA upregulate MMP-2/9, F-actin, and FAK expression to increase FLS migration. Rac1 induces PAK and JNK activation to increase the migration of FLSs. Rac1 also promotes the production of TNF-α, IFN-γ, and IL-17 by T cells. Rac1 and RhoA enhance the anti-apoptotic effects of FLSs via the PI3K/Akt pathway. RhoA/ROCK induces NF-κB activation and IL-23 production in macrophages. Purple, orange, and green arrows indicate molecules mediated by Cdc42, Rac1, and RhoA, respectively. Green boxes indicate common pathogenetic pathways shared by rheumatoid arthritis and osteoarthritis. The left panel demonstrates the mechanisms of four major types of Rho GTPase inhibitors. Spatial regulation inhibitors target the posttranslational modifications of Rho GTPases. GEF interaction inhibitors suppress the activation by Rho GEFs. Nucleotide binding inhibitors prevent nucleotide binding. Downstream effector inhibitors suppress the interaction and activation of downstream effectors (Created with biorender.com). COX, cyclooxygenase; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FLS, fibroblast-like synoviocyte; GEF, guanine nucleotide exchange factors; ICOS, inducible T cell costimulator; IFN, interferon; IL, interleukin; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinases; MMP, matrix metalloproteinase; MRTF-A, myocardin-related transcription factor-A; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa B; NOS, nitric oxide synthase; PAK, p21 activated kinase; PRG, proteoglycan; ROCK, RhoA kinase; ROS, reactive oxygen species; STAT3, signal transducer, and activator of transcription 3; TAZ, PDZ-binding motif; TNC, tenascin C; TNF, tumor necrosis factor; YAP, yes-associated protein.

fibroblast-like synoviocytes (FLSs, type B synoviocytes), which are essential for RA development (Miura and Kanazawa, 2020). The pannus is rich in osteoclasts responsible for bone destruction in RA (Choy, 2012). Studies have reported that Rac1 is involved in the migration of osteoclasts. Osteoclast precursor cell migration is increased by activated Rac1 (Hutami et al., 2017). For the pattern of regulation, Rac1 activation increases the actin polymerization and fusion of osteoclasts, and promotes cell migration (Maruyama et al., 2016). RhoA also exerts similar effects in osteoclasts. RhoA controls cytoskeletal remodeling in osteoclasts via ROCK (Nakano et al., 2019). The effects were confirmed by inhibition of the activation of Rho GTPases. The inhibition of Rac1 and RhoA activities attenuates survival, migration, and bone resorption in differentiated osteoclasts (Lee et al., 2019).

The majority of synoviocytes in the pannus of most RA patients are FLSs (Miura and Kanazawa, 2020). RA FLSs have tumor cell like characteristics, including proliferation, migration, invasion and anti-apoptosis (Tu et al., 2018). FLS migration and invasion into susceptible areas with the secretion of matrix-degrading enzymes cause joint destruction (You et al., 2014). Therefore, RA FLSs are crucial in the pathogenesis and have become a research interest. Studies have indicated that Rho GTPase activation can increase the migrating ability of FLSs. Migrating FLSs exhibit significantly higher levels of Cdc42 expression, which is recruited for cellular movement and offers the potential for migrating to adjacent tissues prone to matrix destruction (Choe et al., 2016). For specific mechanisms, Cdc42 upregulates matrix metalloproteinase (MMP)-2/9, F-actin, and focal adhesion kinase (FAK) expression in FLSs to increase cell migration (Lv et al., 2015). In addition to Cdc42, Rac1 and RhoA promote the invasiveness of RA FLSs (Laragione et al., 2018, 2019; Xiao et al., 2013). Rho GTPases share similar mechanisms in the regulation of migration. Rac1 enhances the expression of MMP-2/9 and p-FAK in RA FLSs to increase migration and invasion (Lv et al., 2015; Niu et al., 2020). In another study, it was reported that the p21 activated kinase (PAK) and c-Jun N-terminal kinase (JNK) pathways were activated by Rac1 to increase RA FLS invasion (Bartok et al., 2014; Chan et al., 2007). Rac1 also increases the activation of phosphoinositide 3-kinase (PI3K) and phosphorylation of Akt, and enhances synovial fibroblast survival, which promotes RA development (Chang et al., 2014; Connor et al., 2006). RhoA promotes the survival of RA synovial cells by enhancing classical RhoA kinase (ROCK)-mediated anti-apoptotic effects (Nagashima et al., 2006). This finding suggests that RhoA functions through signaling pathways other than MMPs and FAK in modulating migration. The increase in F-actin results in polymerization and stress fiber formation, which increases monolayer permeability and impairs barrier function. As Rho GTPases increase the F-actin content, the hyperpermeability of RA FLSs can be decreased by RhoA downregulation, which further suppresses the expression of p38 mitogen-activated protein kinases (MAPK), nuclear factor kappa B (NF-κB), and F-actin and decreases inflammation (Deng et al., 2018).

Angiogenesis

Newly formed blood vessels invade synovial tissues via angiogenesis and facilitate the infiltration of immune cells, which promote the inflammation and destruction of articular cartilage (Elshabrawy et al., 2015). Serum amyloid A, a hepatocyte-synthesized acute phase protein, is increased in RA joints and contributes to RA cell migration and angiogenesis (Connolly et al., 2010; Lv et al., 2016). Studies show that Rho GTPases are associated with serum amyloid A and are involved in angiogenesis. Cdc42 activity is increased by serum amyloid A to induce actin cytoskeleton reorganization in RA synovial fibroblast cells and endothelial cells (Connolly et al., 2011). In contrast, RhoA has no effect on mediating serum amyloid A-induced events (Connolly et al., 2011). Cdc42 and Rac1 are involved in modulating endothelial cell morphology, whereas RhoA is known to regulate endothelial cell branching events; thus, the acute-phase protein serum amyloid A-induced cell migration might be highly dependent on Cdc42 and Rac1-mediated membrane protrusion. Other mechanisms for the regulation of angiogenesis have also been explored by researchers. RhoA-dependent vascular endothelial growth factor (VEGF), Akt, and FAK upregulation in human dermal microvascular endothelial cells enhances angiogenesis, which can lead to RA (Park et al., 2002).

Immune regulation

Rho GTPases are important for macrophage and lymphocyte function, including migration into tissues, inflammatory response, reactive oxygen species (ROS), and cytokine production (Heasman and Ridley, 2008). Rho GTPases have been identified as important regulators of immunity in RA. The T cell costimulatory proteins, inducible T cell costimulator (ICOS), and CD154, are induced by Rac1 activation and are involved in the pathogenesis of RA (Abreu et al., 2010; Rao et al., 2017). Monocytes and macrophages are central to the initiation of inflammatory responses and bone erosion in RA (Davignon et al., 2013). Studies have shown that Rho GTPases are upregulated and regulate a variety of responses in monocytes or macrophages. Rac2 is upregulated in macrophages, and interacts with inducible nitric oxide synthase (iNOS) to produce nitric oxide (NO) along with inflammation in RA synovium (Dey et al., 2016). RhoA activates NF-κB and mediates the secretion of TNF-α and IL-1β in monocytes to enhance the inflammatory response (Lin et al., 2011). In addition, IL-23 production is induced by RhoA/ROCK mediated NF-κB activation in RA macrophages (Xiao et al., 2013). The production of pathogenic autoantibodies in RA is enhanced by RhoB, whereas RhoB deletion alleviates this condition (Mandik-Nayak et al., 2017).

Cytokines are involved in the pathogenesis of RA, and the success of anti-cytokine therapies indicates the critical function of cytokines (Noack and Miossec, 2017). TNF-α, interferon (IFN)-γ and interleukin (IL)-17,

can be activated by Rac1 in T cells (Abreu et al., 2010). Synoviocytes secrete the proinflammatory cytokines IL-1β and IL-6, leading to joint inflammation (Xu et al., 2006). This effect is mediated by RhoA activation, which subsequently mediates tumor necrosis factor alpha (TNF-α)-induced NF-κB activation, as well as the secretion of cytokines (Xu et al., 2006). Blockade of the RhoA effector ROCK also induces NF-κB inactivation and a decrease in proinflammatory cytokines (He et al., 2008; Yokota et al., 2008). These findings indicate that Rho GTPases function as cytokine regulators both directly and indirectly in RA.

Posttranslational modification

Posttranslational modification of Rho GTPases by geranylgeranyltransferase type I (GGTase-I) transferring a 20-carbon geranylgeranyl lipid is deemed to target Rho GTPases for GTP-loading and membrane anchoring (Akula et al., 2019; Samuel and Hynds, 2010). This process is also named prenylation, which is essential for Rho GTPase activation (Samuel and Hynds, 2010). Therefore, inhibiting GGTase-I is reasonable for restricting RA development, and it has been proven in synovial cells via subsequent Rac1 and RhoA inhibition (Chang et al., 2014). However, several reports have indicated the proinflammatory role of GGTase-I inhibition in RA, challenging the view that geranylgeranylation is significant for the function of Rho GTPases. Mice with GGTase-I deficiency are associated with GTP-bound Cdc42, Rac1, and RhoA accumulation in macrophages, with increased ROS and cytokine production, as well as erosive RA development (Khan et al., 2011). Akula et al. demonstrated that in macrophages, nonprenylated Rac1(but not Cdc42 or RhoA) is hyperactivated because of the function of GGTase-I in limiting Rac1 effector interactions and acting as a brake on immune responses (Akula et al., 2019). Nonprenylated Rac1 is highly associated with the adaptor protein Ras GTPase-activating-like protein 1 (Iqgap1), which enhances GTP exchange (Akula et al., 2019). Hence, studies also demonstrate that prenylation inhibition also releases the break and stimulates Rac1-mediated inflammation (Akula et al., 2019). Mechanistically, nonprenylated Rac1-mediated ROS production increases the phosphorylation of the protein kinase Src, signal transducer and activator of transcription 3 (STAT3), and IκB kinase (IKK)α/β in macrophages, thus contributing to inflammation and joint destruction (Akula et al., 2019; Gao et al., 2015; Tas et al., 2006). In addition to prenylation, SUMOylation is another type of posttranslational modification involving the attachment of small-ubiquitin-like modifier (SUMO) family proteins to lysine residues (Wilkinson and Henley, 2010). SUMOylation of Rac1 activates PAK and JNK, which further increases the migration and invasion of RA FLSs, whereas the inhibition of SUMOylation suppresses the aggressive behavior of RA FLSs (Lao et al., 2016, 2019). Compared to prenylation, the role of SUMOylation on Rho GTPases in RA has been less studied and requires further exploration.

Regulation by non-coding RNAs

MicroRNAs (miRNAs) are a class of noncoding RNAs that can regulate gene expression by inhibiting mRNA translation or promoting mRNA degradation (Correia de Sousa et al., 2019). The dysregulation of miRNAs in RA has been widely reported. miRNA-27a suppresses the expression of Rho GTPases, including Cdc42, Rac1, and RhoA, in RA FLSs to inhibit cell migration and invasion (Shi et al., 2016). Targeting Rac1 expression by miRNA-192–5p in rats with collagen-induced arthritis attenuates joint destruction and the inflammatory response (Zheng et al., 2020). Long noncoding RNAs (lncRNAs) are another class of noncoding RNAs longer than 200 nucleotides that regulate various cellular processes (Noh et al., 2018). Zou et al. identified a lncRNA that is expressed at lower levels and involved in the aggressive behavior of RA FLSs, namely LERFS through microarray data (Zou et al., 2018). LERFS interacts with an RNA-binding protein, heterogeneous nuclear ribonucleoprotein Q, and downregulates the mRNA translation of Cdc42, Rac1, and RhoA (Zou et al., 2018). Therefore, the decreased expression of the lncRNA LERFS in RA FLSs contributes to RA pathogenesis by increasing rheumatoid synovial aggression and joint destruction (Zou et al., 2018). In recent years, there has been a growing interest in circular RNAs in RA, whereas their interaction with Rho GTPases remains to be explored.

Osteoarthritis

Osteoarthritis (OA) is a common form of arthritis typically affecting joint cartilage and the underlying bone (Kraus et al., 2015). Articular cartilage degeneration and degradation, as well as subchondral bone sclerosis are characteristics of OA (Yu et al., 2016).

Chondrocytes

Although the pathogenetic mechanisms remain to be explored, in recent years, an increasing number of studies have indicated the involvement of chondrocytes in OA pathogenesis. Dysregulated activation of

Rho GTPases has been observed in OA (Figure 1). Aberrant Rac1 activation has been identified in human OA cartilage (Zhu et al., 2015). Cdc42 signaling pathways are enhanced in chondrocytes prone to promoting OA (Wang et al., 2007). In addition, Cdc42 increases extracellular signal-regulated kinase 1/2 (ERK1/2) activation to activate SMADs, thus leading to subchondral bone remodeling *in vivo* (Hu et al., 2018). Rho GTPases are involved in the destruction of the cartilage matrix. Cdc42 activates PAK and increases the expression of IL-6, which subsequently enhances Janus kinase (JAK)/STAT3 signaling to degrade articular chondrocytes in a mouse OA model (Hu et al., 2018). Rac1 stimulates MMP-13 production and destroys the cartilage matrix in the development of OA (Long et al., 2013). The matrix degradation gene, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-5), and the chondrocyte hypertrophy marker, type X collagen (COLX) are also upregulated by Rac1 in chondrocytes, accelerating OA progression (Zhu et al., 2015). The Rac1/Akt pathway stimulates the phosphorylation of inhibitor of nuclear factor kappa B (IκBα) and degradation, which further enhances NF-κB activity to stimulate the expression of proinflammatory mediators, including nitric oxide synthase 2 (NOS2) and cyclooxygenase 2 (COX2), as well as the collagen degradation proteins, MMP3 and MMP13 (Zhang et al., 2019). The RhoA/ROCK pathway also enhances cartilage damage by collagen degradation (Appleton et al., 2010). Taken together, these studies indicate that Rho GTPases may function as an important regulator of collagen degradation in the pathogenesis of OA.

Mechanical injury is another cause of OA. Excessive chronic or repetitive mechanical loading of cartilage is critical in the pathogenesis of OA, and this effect has been investigated *in vitro* by fluid-induced shear stress in chondrocytes (Jin et al., 2000; Wang et al., 2013). Shear stress in chondrocytes stimulates Rac1 and Cdc42, which activate JNK and further induce MMP-9 expression, which is responsible for collagen degradation (Jin et al., 2000). Gremlin-1 is also induced by excessive mechanical loading through the activation of Rac1 and NF-κB (Chang et al., 2019). Rac1-induced germlin-1 expression further enhances NF-κB activation and MMP expression, and inhibits cartilage matrix genes to mediate cartilage degeneration (Chang et al., 2019). Intriguingly, shear stress increases Rac1 activity in chondrocytes at higher levels, whereas decreases Rac1 activity at lower levels. Reduced expression of Rac1 decreases MMP-13 activity via p38 MAPK signaling, and therefore prevents cartilage degradation (Hamamura et al., 2013). The surgically-induced OA mouse model also demonstrates that knee loading suppresses cartilage destruction (Hamamura et al., 2013). The data indicate that excessive mechanical loading contributes to the pathogenesis of OA partly by modulating Rho GTPases, whereas the application of appropriate mechanical loading could alleviate OA.

Intriguingly, several studies have indicated the protective role of Rho GTPases in OA. RhoB is downregulated in osteoarthritic chondrocytes (Gebhard et al., 2004). Contrary to the aforementioned functions and mechanisms in the pathogenesis of OA, Cdc42 also exerts protective effects. Chondrocyte senescence during aging promotes the development of OA (Jeon et al., 2018). Decreased Cdc42 activation status is identified in aged chondrocytes, indicating that activated Cdc42 maintains the normal phenotype of chondrocytes (Fortier and Miller, 2006). Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) deletion has been demonstrated to restore the phenotype of chondrocytes, and Cdc42 inhibition can reduce the levels of YAP/TAZ (Goto et al., 2018). Cdc42 increases the level of actin formation, which further enhances myocardin-related transcription factor-A (MRTF-A), and subsequently upregulates tenascin C (TNC) expression, which is chondroprotective (Delve et al., 2018). Proteoglycan 4 (PRG4) is also upregulated by Cdc42 and independent of MRTF-A, thus providing protective effects to cartilage (Delve et al., 2018). YAP/TAZ downregulation further leads to the suppression of PRG4 and TNC, indicating that Cdc42 regulates a variety of chondroprotective molecules, which might alleviate OA (Delve et al., 2020). Hence, Cdc42 maintains the normal phenotype and regulates chondroprotective pathways in chondrocytes.

Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) are common genomic variations occurring in a human population, and are correlated with various diseases including OA (Srinivasan et al., 2016; Wang et al., 2016). Genetic studies on Rho GTPases and OA principally focus on RhoB. The polymorphisms of *RHOB* are associated with OA in a European Caucasian population (Mahr et al., 2006). Specifically, the SNPs rs2602160 and rs585017 contribute to OA pathogenesis (Mahr and Müller-Hilke, 2007). Similar effects of the *RHOB* SNP rs585017 have been observed in an East Asian population (Shi et al., 2008). However, Loughlin et al. revealed that the *RHOB* SNP rs585017 is not associated with OA pathogenesis in European Caucasians

(Loughlin et al., 2007). In consideration of the conflicting results, further studies of different ethnic groups and other members of Rho GTPases are needed to elucidate the effects of SNPs in OA.

Systemic sclerosis

Systemic sclerosis (SSc or systemic scleroderma) is a complex disorder involving microvessels, small arteries, and connective tissues, with the deposition of fibrotic tissues and microvascular obliteration in tissues and organs (Barsotti et al., 2019).

Angiogenesis

The loss of angiogenesis is regarded as a critical event in SSc (Matucci-Cerinic et al., 2017). Microvascular endothelial cells lining the vessels are activated during angiogenesis, in which the actin cytoskeleton is important for cell motility and membrane protrusion events (Bayless and Johnson, 2011). Different members of Rho GTPases can induce contrary effects to angiogenesis in SSc. RhoA expression is elevated in microvascular endothelial cells in SSc patients, which skews endothelial cells to an antiangiogenic state by overactivating RhoA/ROCK pathway and suppressing the proangiogenic effect of VEGF (Tsou et al., 2015). In contrast, Cdc42 and Rac1 activity is downregulated in microvascular endothelial cells of patients with SSc resulting in a critical defect in angiogenesis (Margheri et al., 2006). Rac2 inhibition impairs capillary morphogenesis in microvascular endothelial cells, suggesting a promoting role of Rac2 activation in angiogenesis (Giusti et al., 2013).

Fibroblasts

The role of Rho GTPases in SSc pathogenesis is not limited to angiogenesis, but also involves the regulation of fibroblasts. Fibroblast recruitment and activation are early events in SSc (Gilbane et al., 2013). Under exposure to SSc microvascular endothelial cell-conditioned medium, the mesenchymal movement of fibroblasts is increased via the activation of Rac1 and deactivation of RhoA (Serratì et al., 2013). This finding indicates that Rac1 and RhoA have different functions in controlling the movement of fibroblasts. In addition to the movement of fibroblasts, ROS plays an important role in the pathogenesis of scleroderma by enhancing fibrogenesis (Bourji et al., 2015; Dosoki et al., 2017). Rac1 enhances the production of ROS via nicotinamide adenine dinucleotide phosphate (NADPH) in fibroblasts (Napolitano et al., 2018). In addition, Rac1 promotes matrix contraction and the expression of connective tissue growth factor (CTGF), α-smooth muscle actin (α-SMA) and type I collagen in fibroblasts to promote fibrogenesis (Xu et al., 2009). Therefore, suppression of Rac1 might reverse this process. The fibroblast-specific deletion of Rac1 results in resistance to bleomycin-induced cutaneous sclerosis in mice (Liu et al., 2008).

Single nucleotide polymorphisms

Studies from different cohorts have reported the correlation between SNPs in the Rho subfamily members and SSc susceptibility. *RHOA* gene polymorphisms (rs2177268) and *RHOC* gene polymorphisms (rs11102522 and rs11538960) are associated with SSc (Pehlivan et al., 2016). Two *RHOB* SNP loci, namely rs342070 and rs13021401, have been identified to increase the risk of SSc in European patients (Allanore et al., 2011). However, in another study among patients from several European countries and the USA, polymorphisms in *RHOB* (rs342070 and rs13021401) showed no significant associations with SSc (Bossini-Castillo et al., 2013). *RHOB* polymorphisms have been observed in Chinese Han SSc patients, and the rs1062292 locus is associated with susceptibility to SSc (Shu et al., 2014). The association between SNPs and susceptibility to SSc remains controversial, and the predictive value is still restricted.

Other rheumatic diseases

Rho GTPases are involved in other rheumatic diseases, such as systemic lupus erythematosus (SLE) and Sjögren's syndrome. SLE is characterized by the activation of the interferon system and inflammatory cytokines (Bengtsson and Rönnblom, 2017; Ohl and Tenbrock, 2011). The pathogenesis of SLE involves plasmacytoid dendritic cells (pDCs), which produce interferons and have a profound effect on autoimmunity (Huang et al., 2015). Rac1 activation leads to the activation of IKKα in pDCs, which stimulates IFN-α production in SLE pathogenesis (Chu et al., 2019; Rozo et al., 2017). RhoA-ROCK pathway enhances the production of IL-17 and IL-21 in SLE T cells or human Th17 cells (Rozo et al., 2017). Intriguingly, a study has reported the potential anti-inflammatory role of RhoA in lupus T cells (Fan et al., 2012). The defined role of Rho GTPases in other rheumatic diseases requires further investigation.

TARGETED THERAPY

Generally, Rho GTPases play an important role in deteriorating rheumatic diseases including RA, OA, and SSc (Table 1). Consequently, targeting Rho GTPases with inhibitors, including inhibition of GEF interactions, decreased nucleotide binding, suppression of downstream effectors and restriction of spatial regulation might be an attractive and promising choice to delay disease progression, and even reverse the conditions (Table 2).

The functions of Rho GTPases are highly dependent on their activities rather than their expression, and therefore inhibitors of Rho GEFs are most widely used in preclinical studies. The Cdc42 inhibitor ZCL278, which blocks GEF binding and deactivates Cdc42, alleviates articular cartilage degradation and subchondral bone remodeling via inhibition of Cdc42 (Friesland et al., 2013; Hu et al., 2018). ML141, another potent and selective Cdc42 inhibitor via nucleotide-binding blockade, reduces the expression of MRTF-A in superficial zone chondrocytes, which might further decrease the expression of protective molecules for OA, including PRG4 and TNC (Delve et al., 2018, 2020; Surviladze et al., 2010).

NSC23766 is the first developed and most widely used Rac1 GEF inhibitor in research (Gao et al., 2004). Current evidence for NSC23766 mainly focuses on changes in RA cells. The Rac1 inhibitor NSC23766 abrogates serum amyloid A-induced actin cytoskeleton reorganization in RA cells, and therefore reduces cell migration, invasion, and angiogenesis (Connolly et al., 2011). NSC23766 suppresses IL-17A-induced Rac1 activation in RA synovial fibroblasts, thus leading to decreased cell migration and invasion (Moran et al., 2011). In addition, NSC23766 reduces MMP-2/p and p-FAK expression via Rac1 inhibition in RA FLSs, and decreases cell migration and invasion (Niu et al., 2020). *In vivo* studies have shown that NSC23766 also alleviates collagen-induced arthritis, which is represented by reduced ankle stiffness, hind paw thickness, arthritic scores and incidence in mice (Chen, 2020). For SSc, NSC23766 is reported to suppress the migration and collagen protein expression of SSc fibroblasts (Xu et al., 2009).

Recent advances in biomaterials offer new opportunities for therapy. For example, studies indicate that chitosan and hydrogels improve the effects of Rho GTPase inhibitors. Chitosan microspheres are used to provide controlled drug release and improve drug bioavailability (Mitra and Dey, 2011). A novel strategy of controlled release of NSC23766 by chitosan microspheres was developed, and chitosan microspheres were injected into the OA knees of a surgically induced OA mouse model, which delayed OA development and showed promise for clinical practice (Zhu et al., 2015). Local administration of a Rac1 inhibitor also avoids a variety of side effects compared to systemic administration. Recently, researchers have developed a novel alginate-derived hydrogel system containing the potent RhoA inhibitor, *C. botulinum* C3 transferase, for sustained inhibition of RhoA (Formica et al., 2018). The implantation of the hydrogel *in vivo* increases the production of collagen-rich matrix and retention (Formica et al., 2018). Future research could focus on the targeted delivery of Rho GTPase inhibitors by bioengineered materials.

Compounds derived from traditional medicine for rheumatic diseases might show beneficial effects. Tetrandrine is a natural compound extracted from the Chinese herb *Stephania tetrandra*, which is used for the treatment of rheumatic diseases (Seow et al., 1988). Tetrandrine can reduce the expression of Cdc42, Rac1, and RhoA in FLSs, decreasing their migration (Lv et al., 2015).

Research on lipid-lowering drugs demonstrates promising inhibitory effects on Rho GTPases. Fluvastatin is a lipid-lowering drug showing therapeutic potential in RA patients (Hegazy et al., 2016). The cholesterol-independent effects of statins indicate that the geranylgeranyl pyrophosphate, which attaches lipids for the posttranslational modification of various molecules, is blocked by statins (Nagashima et al., 2006). Fluvastatin was first found to increase the apoptosis of RA synovial cells by decreasing the membrane fraction and increasing the cytosolic fraction of RhoA, and this effect is achieved by the inhibition of geranylgeranylation (Nagashima et al., 2006). The geranylgeranylated membrane fraction of both RhoA and Rac1 can be inactivated by Fluvastatin, subsequently leading to PI3K/Akt inhibition and the apoptosis of synovial cells (Akula et al., 2019). Simvastatin is another type of lipid-lowering drug belonging to statins. The administration of simvastatin suppresses RhoA activation via geranylgeranylation inhibition in RA synoviocytes, and further decreases the secretion of pro-inflammatory cytokines (Xu et al., 2006). The RhoA-mediated migration, adhesion, and invasion are inhibited by simvastatin in RA FLSs (Xiao et al., 2013). In macrophages, the activation of pro-inflammatory signaling is also suppressed by simvastatin, which attenuates the inflammatory response of RA (Lin et al., 2011). For SLE, simvastatin decreases the production of IL-17

Table 1. Effects of Rho GTPases in rheumatic diseases

Rho GTPase	Effect	Downstream mechanism	Models	Modulation	Reference
Rheumatoid arthritis					
Cdc42	Cdc42 expression is enhanced in migrating RA FLSs, leading to increased cell movement.	/	*in vitro*	Upstream inhibition	Choe et al. (2016)
Cdc42 Rac1	Cdc42, but not RhoA, induces cytoskeletal reorganization in RA synovial fibroblasts and microvascular endothelial cells. Cdc42/Rac1 inhibition abrogates serum amyloid A-induced cell growth, migration, invasion, and angiogenesis.	/	*in vitro*	Upstream inhibition Inhibitor	Connolly et al. (2011)
Cdc42 Rac1 RhoA	Cdc42, Rac1, and RhoA accumulation in macrophage is increased under GGTase-I. Cdc42, Rac1, and RhoA accumulation in macrophage initiates rheumatoid arthritis.	/	*in vitro*	Knock-down (shRNA) Prenylation inhibition	Khan et al. (2011)
	Cdc42, Rac1, and RhoA upregulate MMP-2/9, F-actin and FAK expression to increase RA FLS migration.	Cdc42, Rac1, RhoA–MMP-2/9, F-actin, FAK	*in vitro*	Upstream inhibition	Lv et al. (2015)
	Cdc42, Rac1, and RhoA expression can be downregulated by miRNA-27a in RA FLSs. Cdc42, Rac1, and RhoA downregulation inhibits cell migration and invasion.	/	*in vitro*	miRNA	Shi et al. (2016)
	Cdc42, Rac1, and RhoA expression can be downregulated by lncRNA LERFS in RA FLSs to decrease migration.	/	*in vitro*	lncRNA	Zou et al. (2018)

(*Continued on next page*)

Table 1. *Continued*

Rho GTPase	Effect	Downstream mechanism	Models	Modulation	Reference
Rac1	Rac1 increases phosphorylated Akt levels and enhances synovial fibroblast viability.	Rac1–Akt	*in vitro*	Knock-down (siRNA)	Connor et al. (2006)
	Rac1 enhances JNK activation and increases RA FLS invasion.	Rac1–JNK	*in vitro*	Knock-down (siRNA) Inhibitor	Chan et al. (2007)
	Rac1 deletion suppresses TNF-α, IFN-γ and IL-17 by T cells, and the costimulatory protein ICOS and CD154 for B-cell help.	Rac1–TNF-α, IFN-γ, IL-17, ICOS, CD154	*in vivo*	Inhibitory peptide	Abreu et al. (2010)
	Rac1 increases the migration and invasion of synovial fibroblasts.	/	*in vitro*	Upstream inhibition	Bartok et al. (2014)
	Rac1 SUMOylation induces PAK and JNK activation, increasing the migration of RA FLSs.	Rac1–PAK/JNK	*in vitro*	Upstream inhibition Inhibitor	Lao et al. (2016)
	Rac1 inactivation impairs the actin polymerization and fusion of osteoclasts.	/	*in vitro*	Upstream inhibition	Maruyama et al. (2016)
	Rac1 activation in osteoclast precursor cells increases the migratory behavior.	/	*in vivo*	Upstream inhibition	Hutami et al. (2017)
	Rac1 activation increases the invasiveness of RA FLSs.	/	*in vitro*	Upstream inhibition	Laragione et al. (2018)
	Inhibiting prenylation enhances Rac1-effector interactions and unleashes proinflammatory signaling. Rac1-mediated ROS production increases the phosphorylation of Src, STAT3, and IKKα/β in macrophages. Rac1 increases p38 activation via a ROS-independent pathway.	Rac1–ROS–Src, STAT3, IKKα/β Rac1–p38	*in vitro* *in vivo*	Knock-out Prenylation inhibition Upstream inhibition	Akula et al. (2019)
	Rac1 SUMOylation increases Rac1 activation and the aggressive behavior of RA FLSs.	Rac1–PAK	*in vitro*	SUMOylation inhibition	Lao et al. (2019)
	Rac1 increases RA FLS migration and invasion via increasing the expression of MMP-2, MMP-9, and p-FAK.	Rac1–MMP-2/9, p-FAK	*in vitro* *in vivo*	Inhibitor	Niu et al. (2020)

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Table 1. *Continued*

Rho GTPase	Effect	Downstream mechanism	Models	Modulation	Reference
Rac1 RhoA	Rac1 activation enhances actin polymerization in osteoclasts and promotes migration.	/	*in vitro*	Upstream inhibition	Lee et al. (2019)
Rac2	Rac2 downregulation decreases joint destruction, inflammatory response, and clinical arthritic scores in rats with collagen-induced arthritis.	/	*in vitro* *in vivo*	miRNA	Zheng et al. (2020)
RhoA	RhoA promotes anti-apoptotic effects in RA synovial cells via the effector ROCK.	RhoA–ROCK	*in vitro*	Inhibitor	Nagashima et al. (2006)
	RhoA promotes inflammatory cytokine secretion in rheumatoid synoviocytes via TNF-α-induced NF-κB activation.	RhoA–TNF-α–NF-κB–IL-1β, IL-6	*in vitro*	Dominant-negative Mutant	Xu et al. (2006)
	RhoA mediates NF-κB activation and the secretion of TNF-α and IL-1β in monocytes.	RhoA–NF-κB, TNFα, IL-1β	*in vitro*	Inhibitor	Lin et al. (2011)
	RhoA increases the migration, adhesion and invasion of RA FLSs.	/	*in vitro*	Inhibitor	Xiao et al. (2013)
	RhoA increases the migration, adhesion and invasion of RA FLSs.	RhoA–ROCK–NF-κB–IL-23	*in vitro*	Inhibitor Mutant	Park et al. (2013)
	RhoA mediates actin cytoskeletal remodeling in osteoclasts.	RhoA–ROCK2	*in vitro*	Upstream inhibition	Nakano et al. (2019)
Osteoarthritis					

(*Continued on next page*)

Table 1. *Continued*

Rho GTPase	Effect	Downstream mechanism	Models	Modulation	Reference
Cdc42	Cdc42 deactivation is protective for aged chondrocytes.	/	*in vitro*	Upstream inhibition Mutant	Fortier and Miller (2006)
	Cdc42 increases the expressions of TNC and PRG4 in chondrocytes, which are protective to the cartilage.	Cdc42–Actin–MRTF-A–TNC Cdc42–PRG4	*in vitro*	Inhibitor	Delve et al. (2018)
	Cdc42 inhibition attenuates OA development. Cdc42 activates PAK to induce IL-6 production and activates JAK/STAT3 pathway to induce articular degeneration. Cdc42 increases ERK1/2 to activate SMADs in subchondral bone remodeling.	Cdc42–PAK–IL-6–JAK–STAT3 Cdc42–ERK1/2–SMADs	*in vitro* *in vivo*	Inhibitor Knock-out	Hu et al. (2018)
	Cdc42 inhibition downregulates YAP/TAZ and decreases TNC and PRG4 expressions, which are chondroprotective.	Cdc42–YAP/TAZ–TNC, PRG4	*in vitro*	Inhibitor	Delve et al. (2020)
Rac1	Rac1-mediated p38 MAPK signaling increases MMP-13 activity and promotes cartilage degradation.	Rac1–p38 MAPK–MMP-13	*in vivo*	Knock-down Mutant	Hamamura et al. (2013)
	Rac1 increases MMP-13 production in chondrocytes.	Rac1–MMP-13	*in vitro*	Inhibitor Knock-down (siRNA) Mutant	Long et al. (2013)
	Rac1 aberrant expression is identified in OA cartilage, and promotes MMP-13, ADAMTS-5, and COLX expressions in chondrocytes	Rac1–β-catenin–MMP-13, ADAMTS-5, COLX	*in vivo*	Upstream inhibition Mutant	Zhu et al. (2015)
	Rac1 activation enhances ROS production and downstream signaling, leading to osteoarthritis.	Rac1–ROS–Gremlin-1–NF-κB–MMP	*in vivo*	Inhibitor	Chang et al. (2019)
	Rac1 aggravates cartilage degradation in OA via the activation of Akt and NF-κB pathway.	Rac1–Akt–IκBα–NF-κB–MMP3/13, NOS2, COX2	*in vivo*	Knock-down (siRNA)	Zhang et al. (2019)

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Table 1. *Continued*

Rho GTPase	Effect	Downstream mechanism	Models	Modulation	Reference
RhoA	RhoA/ROCK pathway inhibition reduces the degradation of collagen.	RhoA–ROCK	*in vitro*	Inhibitor	Appleton et al. (2010)
RhoB	SNPs are associated with disease susceptibility.	/			Mahr et al. (2006) Mahr and Müller-Hilke (2007) Shi et al. (2008)
	SNP (rs585017) is not associated with disease susceptibility.	/			Loughlin et al. (2007)
Systemic sclerosis					
Cdc42 Rac1	Cdc42 and Rac1 inactivation inhibits cytoskeletal rearrangements and cell motility of endothelial cells.	/	*in vitro*	Upstream inhibition	Margheri et al. (2006)
Rac1	Rac1 enhances NADPH-mediated production of ROS in fibroblasts.	Rac1–NADPH–ROS	*in vitro*	Upstream modulation	Napolitano et al. (2018)
Rac1 RhoA	Rac1 activation and RhoA inhibition are required for the mesenchymal motility of fibroblasts.	/	*in vitro*	Upstream inhibition	Serratì et al. (2013)
Rac2	Rac2 inhibition results in the impairment of capillary morphogenesis in microvascular endothelial cells.	/	*in vitro*	Knock-down (siRNA)	Giusti et al. (2013)
RhoA	RhoA is upregulated in SSc microvascular endothelial cells.	/	*in vitro*	Upstream modulation	Tsou et al. (2015)
RhoA RhoB RhoC	SNPs are associated with disease susceptibility.	/			Pehlivan et al. (2016) Allanore et al. (2011) Shu et al. (2014)
RhoB	SNPs (rs342070 and rs13021401) are not associated with an increased risk for SSc.	/			Bossini-Castillo et al. (2013)

FLS, fibroblast-like synoviocyte; GGTase-I, geranylgeranyltransferase type I; ICOS, inducible T-cell costimulator; IFN, interferon; IKK, IκB kinase; IL, interleukin; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; lncRNA, long noncoding RNA; MAPK, mitogen-activated protein kinases; miRNA, microRNA; MMP, matrix metalloproteinase; MRTF-A, myocardin-related transcription factor-A; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa B; NOS, nitric oxide synthase; OA, osteoarthritis; PAK, p21 activated kinase; PRG, proteoglycan; RA, rheumatoid arthritis; ROCK, RhoA kinase; ROS, reactive oxygen species; shRNA, short hairpin RNA; siRNA, small interfering RNA; SNP, SNP SSc, systemic sclerosis; STAT3, signal transducer and activator of transcription 3; SUMO, small-ubiquitin-like modifier; TAZ, PDZ-binding motif; TNC, tenascin C; TNF, tumor necrosis factor; YAP, yes-associated protein.

Table 2. Targeted therapy of Rho GTPases in rheumatic diseases

Disease	Models	Rho GTPase	Inhibitor	Concentration	Effect	Reference
Rheumatoid arthritis	Human dermal endothelial cell Rheumatoid arthritis synovial fibroblasts	Rac1	NSC23766	50 μM (*in vitro*)	Rac1 blockade inhibits cell growth, actin cytoskeleton rearrangement, invasion, migration and angiogenesis.	Connollyet al. (2011)
	Rheumatoid arthritis fibroblast-like synoviocytes	Rac1	NSC23766	50 μM (*in vitro*)	Rac1 inhibition reduces RA FLS invasion.	Chan et al. (2007)
	Rheumatoid arthritis synovial fibroblasts	Rac1	NSC23766	50 μM (*in vitro*)	Rac1 inhibition suppresses RA synovial fibroblast migration and invasion.	Moran et al. (2011)
	Rheumatoid arthritis fibroblast-like synoviocytes	Rac1	NSC23766	/	Rac1 inhibition decreases the migration and invasion of RA FLSs.	Lao et al. (2016)
	Rheumatoid arthritis fibroblast-like synoviocytes	Rac1	NSC23766	/	Rac1 inhibition suppresses the aggressive behavior of RA FLSs.	Lao et al. (2019)
	Rheumatoid arthritis fibroblast-like synoviocytes	Rac1	NSC23766	/	Rac1 inhibition suppresses the invasiveness of RA FLSs.	Lao et al. (2019)
Osteoarthritis	Superficial zone chondrocytes	Cdc42	ML141	10 μM (*in vitro*)	Cdc42 inhibition decreases actin polymerization status and reduces the expression of superficial zone molecules.	Delve et al. (2018)
	Mouse primary articular chondrocytes Mouse embryonic fibroblast C3H10T1/2 cells Mouse chondrogenic ATDC5 cells	Cdc42	ZCL278	20 to 50 μM (*in vitro*) 8 μg/knee joint (*in vivo*)	Cdc42 inhibition alleviates articular cartilage degeneration and subchondral bone deterioration of osteoarthritis.	Hu et al. (2018)
	Superficial zone chondrocytes	Cdc42	ML141	10 μM (*in vitro*)	Cdc42 inhibition reduces the expression of chondroprotective molecules.	Delve et al. (2020)
	Human articular chondrocytes	Rac1	NSC23766 EHT1864	100 μM (*in vitro*) 25 μM (*in vitro*)	Rac1 inhibition reduces the production of enzymes degrading the cartilage.	Long et al. (2013)
	Human articular chondrocytes	Rac1	NSC23766	50 μM (*in vitro*) 30 μg/site (*in vivo*)	Rac1 inhibition protects cartilage from destruction. Controlled release of Rac1 inhibitors is achieved by chitosan microspheres.	Zhu et al. (2015)
	Mouse chondrogenic ATDC5 cells	Rac1	NSC23766 EHT1864	10 to 100 μM (*in vitro*) 10 to 100 μM (*in vitro*)	Rac1 inhibition suppresses Gremlin-1 signaling.	Chang et al. (2019)
Systemic sclerosis	Human dermal fibroblasts	Rac1	NSC23766	50 mM (*in vitro*)	Rac1 inhibition reverses the phenotype of fibrotic fibroblasts.	Xu et al. (2009)

FLS: fibroblast-like synoviocyte; RA: rheumatoid arthritis; OA: osteoarthritis.

and IL-21 in SLE T cells or Th17 cells (Rozo et al., 2017). The results indicate that the patients with RA theoretically could benefit from statins.

Observational studies have demonstrated that the risk of developing RA is reduced under persistent statin therapy (Chodick et al., 2010). However, increased risk of RA is also observed among statin users in other studies (de Jong et al., 2012). A recent systematic review and meta-analysis of 8 observational studies shows that the risk of RA might be lower in patients with higher than lower persistence or intensity of statin treatment, whereas no difference is observed for RA risk in statin users compared to that in non-users (Myasoedova et al., 2020). The effect of statin in rheumatic diseases clinically remains controversial, and large-scale, well designed observational studies as well as randomized controlled trials are warranted.

It is well known that the functions of Rho GTPases largely depend on the activation of Rho GTPases, whereas inactive ones sparely exert effects. From this perspective, it is more effective to target GEFs for disrupting the events in the development of rheumatic diseases. Upon activation, the regulation of cell behavior by Rho GTPases is also determined by downstream effectors. Compared to upstream signaling, downstream effector pathways are better understood, thus the development of small molecule inhibitors targeting effectors is more successful and promising (Clayton and Ridley, 2020; Lin and Zheng, 2015).

CONCLUSIONS AND FUTURE DIRECTIONS

Emerging studies have indicated that Rho GTPases are involved in the pathogenesis of rheumatic diseases. A large majority of the aforementioned studies demonstrate the detrimental role of Rho GTPases in rheumatic diseases. The upregulation and activation of Rho GTPases in various cell types, such as fibroblasts, chondrocytes, synoviocytes, and immunocytes can promote disease progression both *in vitro* and *in vivo*. It should be noted that several studies have demonstrated the protective roles of Rho GTPases in rheumatic diseases, thus indicating that their effects can be context-dependent.

In recent years, advances in omics studies, including genomics, epigenomics, metabolomics, and proteomics have provided elaborate information on dysregulated gene expression profiles in rheumatic diseases. For example, compared with the gene expression microarray data of synovium, whole blood cells, CD4+ T cells and peripheral blood mononuclear cells from healthy controls, those from patients with RA demonstrate significant activation of Cdc42 signaling pathways (Lee et al., 2020). Proteomics data from synovial fluid of the osteoarthritic knee demonstrate decreased expression of Rho GDP Dissociation Inhibitor Beta (ARHGDIB) than that of the healthy knee, which might indicate enhanced activation of Rho GTPases (Ritter et al., 2013). Single-cell RNA sequencing (scRNA-seq) studies are emerging to reveal the development, progression, and treatment-response patterns of rheumatic diseases (Ji et al., 2019; Lewis et al., 2019). Future research could utilize *in silico* methodologies to analyze the published data and provide in-depth analyses.

However, current studies on Rho GTPases and their regulators in rheumatic diseases mainly involve *in vitro* and *in vivo* data, and are limited by the lack of bulk and patient-level data. Although molecular mechanisms related to Rho GTPases in conditions mimicking rheumatic diseases have been identified, their complex etiology is difficult to be represented by the alterations in cell and animal models. This limitation hinders the ability to directly establish the connection between Rho GTPases and rheumatic diseases and impairs the interpretation of the findings.

Because Rho GTPases generally promote the pathogenesis of rheumatic diseases, inhibitors targeting Rho GTPases are considered as a promising therapeutic strategy. The inhibition of Rho GTPases could not only alleviate the diseases, but also relieve their complications. However, Rho GTPase inhibitors are not currently available in clinical practice for any disorders. Because of the extensive involvement of Rho GTPases in basic fundamental processes, such as glucose uptake, immune response, and organ development, general inhibition of Rho GTPases would exert unexpected and even detrimental effects on physiological activities (Zeng et al., 2020). Recent developments in biotechnologies, including liposomes, exosomes, nanoparticles, and polymeric micelles, provide promising modalities for targeted therapy of rheumatic diseases (Feng and Chen, 2018). Both enhancing the efficacy and minimizing the adverse effects of Rho GTPases are the priorities for future research on Rho GTPase inhibitors.

Several concerns associated with Rho GTPases in rheumatic diseases still need to be addressed. Most importantly, patient-level data are needed to confirm the effects of Rho GTPases in rheumatic diseases. More specific mechanisms by which Rho GTPases contribute to the dysfunction of cells remain to be explored. Inhibitors with higher potency can be used to achieve better inhibitory effects, especially for *in vivo* studies (Montalvo-Ortiz et al., 2012). Meanwhile, the side effects accompanied by the use of inhibitors should be evaluated (Tcherkezian and Lamarche-Vane, 2007). Additional preclinical studies in different models are needed before the application of inhibitors in clinical practice.

In summary, Rho GTPases are implicated in the pathogenesis of rheumatic diseases, and patient-level data and in-depth investigations are needed to validate their functions. Targeted therapy using Rho GTPase inhibitors is promising for the treatment of rheumatic diseases, whereas the effectiveness and safety should be further evaluated preclinically.

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AUTHOR CONTRIBUTIONS

RZ, ZZ, YL - Drafting and revision of the manuscript; WS, HC – Supervision, revision, and obtained funding.

DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest.

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