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# *Advances in signaling pathways and targets of fibroblast-like synoviocytes in rheumatoid arthritis*

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***Abstract:*** Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease significantly characterized by the abnormal proliferation and activation of fibroblast-like synoviocytes (FLS) in the synovial membrane. Studies have shown that FLS play an important role in the development and are involved in pathological processes such as inflammatory response, bone destruction and synovial proliferation. In this paper, we review the recent research progress of FLS signaling pathways, including inflammation-related signaling pathways, apoptosis and survival signaling pathways, and cell migration and invasion signaling pathways. In addition, we discuss the potential therapeutic targets of FLS signaling pathways. By discussing these research advances in depth, we hope to provide new ideas and directions for the treatment of RA.

## 1. Introduction

Rheumatoid arthritis (RA) is a common chronic autoimmune disease that mainly affects the joints and causes pain, limitation of movement, and inflammatory response. A key component in the development of RA is the abnormal activation and proliferation of FLS, which play an important role in RA joint pathology. In recent years, more and more studies on RA-FLS have been conducted, mainly focusing on revealing their signaling pathways and potential therapeutic targets. RA-FLS are unique cell types within the synovium, exhibiting high epigenetic heterogeneity and functional diversity. They are not only involved in pathological processes such as articular cartilage destruction, synovial proliferation and invasion, but also regulate the inflammatory response by producing a variety of inflammatory mediators [1]. Therefore, understanding and intervening in the aberrant signaling pathways of RA-FLS is crucial for the development of new therapeutic strategies and drugs. Researchers are committed to exploring the signaling pathways that influence key pathological processes such as RA-FLS proliferation, invasion, inflammatory response and synovial hyperplasia, and summarizing the latest research targets.

## 2. Biological characteristics of RA-FLS

FLS are highly specialized mesenchymal cells found in the synovium of bicompartmental joints. Normal synovial fibroblasts are mainly distributed in the synovial lining layer, secrete appropriate synovial fluid to reduce bone friction and joint damage, and secrete a variety of cytokines to nourish the joints and to ensure the normal progression of joint activities. In RA, FLS exhibit a variety of biological characteristics, including abnormal proliferation, high aggressiveness, and overproduction of inflammatory factors and cytokines. RA-FLS proliferation rate is significantly increased, leading to overproliferation of synovial tissue and joint destruction. At the same time, their highly invasive ability to penetrate and destroy articular cartilage and bone tissue leads to joint deformity and loss of function [2].

In addition, RA-FLS are involved in the production and maintenance of synovial inflammatory responses by enhancing the secretion of inflammatory factors and cytokines [3,4]. Overproduction of inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) leads to synovial inflammation and joint destruction. RA-FLS also produces a variety of cytokines such as vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), which promote synovial angiogenesis and cell migration. At the same time, RA-FLS immunomodulatory function is altered and participates in the regulation of immune response by interacting with immune cells such as T cells. This makes RA-FLS an important mediator of inflammatory cell aggregation and immune response [5]. These biological features play an important role in the development of RA.

## 3. RA-FLS signaling pathways

### 3.1 RA-FLS signaling pathways

RA-FLS is involved in the regulation of inflammatory responses through multiple signaling pathways. Among them, the Toll-like receptor (TLR) signaling pathway is one of the most important inflammation-related signaling pathways [6].TLR family members include TLR2, TLR4, etc., which are able to sense the molecular patterns of bacteria, viruses, and other pathogens, which in turn initiates inflammatory responses. When TLRs bind to their ligands, they activate downstream signaling molecules. For example, as a response to bacterial peptidoglycan, TLR-2 may be activated in RA-FL and play an important role in the chemotaxis of immune cells towards arthritic joints and the development of synovitis by inducing the production of pro-angiogenic factors such as VEGF and IL-8 [7].

In addition to the TLR signaling pathway, the NF-κB signaling pathway also plays an important role in the upregulation of inflammation in RA-FLS. On the one hand, highly active NF-κB triggers the production of multiple pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6. The upregulation of these pro-inflammatory cytokines regulates the activation of NF-κB through a positive feedback mechanism, creating a vicious cycle. For example, TNF-α initiates the transcription of anti-apoptotic factor genes, such as Bcl-2, nitric oxide synthase, and apoptosis inhibitory proteins, through the over-activation of NF-κB, thereby resisting death receptor-induced apoptosis and accelerating the progression of RA [8]. On the other hand, overactivation of NF-κB also leads to abnormal apoptosis of FLS, which is the main cause of synovial hyperplasia in RA. Abnormal apoptotic FLS further aggregates and adheres to cartilage and bone, exacerbating the destruction of articular cartilage and bone [9]. p38 mitogen-activated protein kinase (p38 MAPK) is an important intracellular signaling pathway involved in the regulation of a number of biological processes, including cell proliferation, apoptosis, inflammation, differentiation, migration and invasion [10]. The role of the p38 MAPK signaling pathway has been gradually emphasized during the occurrence and development of RA. Recent studies have found that the blocking effects of p38

inhibitors on TNF-α, IL-1β, MMP-1, MMP-3, IL-6, and IL-8 may regulate the IL-17 signaling pathway and the release of pro-inflammatory cytokines and mediators [11].

### 3.2 Apoptosis and survival signaling pathways

Apoptosis and survival in RA-FLS are regulated by several signaling pathways. The Bcl-2 family including anti-apoptotic factors (e.g., Bcl-2, Bcl-xL) and pro-apoptotic factors (e.g., Bax, Bad) influences the process of apoptosis by regulating mitochondrial membrane permeability [12]. In RA-FLS, an imbalance of the Bcl-2 family leads to inhibition of apoptosis, which contributes to the development of RA [13]. Increasing evidence suggests that anti-apoptotic proteins, such as Bcl-2, Mcl-2 and FLICE inhibitory protein (FLIP) are upregulated in the FLS of RA patients, whereas pro-apoptotic proteins, such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), p53 up-regulated apoptosis factor (PUMA), and recombinant human BH3 structural domain apoptosis-inducing protein (BID) are downregulated in the RA patients with FLS were downregulated [14]. In addition, an increase in p53 mutants and a relative decrease in p53 expression have also been reported in patients with RA-FLS, which is considered to be one of the important reasons for the excessive proliferation and inadequate apoptosis in FLS [14,15]. Secondly, the mitochondrial regulatory pathway plays an important role in apoptosis and survival of RA-FLS. Mitochondria are intracellular energy centers and key regulators of apoptosis. After RA-FLS is stimulated by inflammatory factors, the mitochondrial membrane potential decreases, leading to a shift in mitochondrial permeability and release of apoptosis-associated proteins, which ultimately triggers the process of apoptosis [16].

The role played by the PI3K/AKT signaling pathway in the apoptosis and survival of RA-FLS should not be overlooked. Activation of the PI3K/AKT signaling pathway inhibits apoptosis and promotes proliferation and survival of FLS. Recent studies have found that signalin 5A further enhances the survival of RA-FLS and promotes its inflammatory and invasive properties by decreasing apoptosis and inhibiting iron death in FLS cells, thereby exacerbating the destructive inflammatory and immune microenvironment by a mechanism that may involve the activation of the PI3K/AKT/mTOR signaling pathway and its downstream molecules [17]. Phosphorylation of PI3K kinase generates PIP3, which in turn activates AKT protein kinase, which ultimately regulates the expression and function of apoptosis-related proteins, thereby protecting RA-FLS from apoptosis. The PI3K/AKT signaling pathway is not only involved in abnormal proliferation and apoptosis inhibition of FLS cells, but also affects osteoclast differentiation and generation. Cai [18] showed that FLS-secreted SPP1 promotes collagen production through PI3K/ AKT signaling to promote osteoclast formation in collagen-induced arthritis, and the Spp1 gene in FLS is expected to be a marker for RA diagnosis.

### 3.3 Cell migration and invasion signaling pathways

Multiple signaling pathways are involved in regulating the migration and invasion process of RA-FLS. The extracellular matrix (ECM) plays an important role by providing physical support and signaling molecules. Among them, fibronectin enhances the inflammatory response in RA-FLS by activating the TLR4/NFκB pathway, and recombinant human peptidylate deiminase (PAD)-activated fibronectin increases RANKL expression and exacerbates disease progression [19]. In addition, RA-FLS promotes migration and invasion by degrading proteases of the ECM (e.g., MMPs) and is involved in joint destruction and synovial thickening. For example, FLS plays a key role in the development of RA vascular opacities, an inflammatory and aggressive synovial tissue responsible for erosive damage in advanced RA. FLS within the vascular pimple degrades MMPs and digests various proteins in cartilage and supporting structures, allowing further expansion and

invasion of the vascular pimple [20].

Integrins are a class of receptors present on the cell surface that interact with proteins in the extracellular matrix (ECM) and activate downstream signaling pathways. Immunohistochemistry experiments by Xu et al. confirmed that the expression of SLC3A2 and integrin β3 was higher in RA synovial tissues than in OA tissues. By inhibiting the expression of SLC3A2/integrin β3 and its downstream signaling molecules, the migration and proliferation of RA-FLS could be inhibited. This reveals the important role of integrins in regulating cell migration and proliferation in RA-FLS [21]. The Rho GTPase family has been reported to play an important role in the regulation of migration and invasion in RA-FLSs. Among them, RND3, a member of the superfamily of GTPase proteins, inhibits NF-kB activation and pro-inflammatory cytokine production by blocking Rho-kinase activation, thereby suppressing the development of RA synovitis. Reducing RhoA levels also effectively inhibits the invasive ability of RA-FLS [22].

## 4. Progress in the study of RA-FLS targets

Researchers are constantly exploring new targets to intervene in the abnormal function of FLS. Some of the novel drug targets include miRNAs, protein kinases, and others. These targets have been investigated with the aim of modulating the inflammatory response, proliferation, and invasive capacity of FLS and improving the symptoms of RA. miRNA target research is an area of great interest and has made some progress in recent years. miRNAs are a class of non-coding RNA molecules of approximately 22 nucleotides in size that regulate gene expression by binding to mRNA targets [23]. miR-155 is widely recognized to play an important role in RA inflammation. Several studies have shown that miR-155 promotes the invasive and inflammatory responses in RA-FLS by targeting and regulating genes such as SOCS1, SHIP1, and FOXO3a [24,25]. miR-146a has been found to be up-regulated in RA-FLSs, and its expression level is closely correlated with the levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), and plays a regulatory T cell inhibitory function [26]. miR-146a regulates the invasive and proliferative capacity of RA-FLSs by inhibiting signaling pathways such as pSTAT-1, IL-6 and TNF-α [27].

Pain is a manifestation mediated by both inflammatory and non-inflammatory mechanisms, and the study of its associated mechanisms can promote individualization and precision of treatment, improve therapeutic efficacy, and reduce pain and discomfort in patients. PTEN is an important target of pro-inflammatory cytokines, which can modulate the inflammatory state in order to alleviate the symptoms of arthritis. PTEN was found to inhibit STAT3 activation and improve the degree of inflammation, bone damage and cartilage damage in mice with collagen-induced arthritis by administering PTEN treatment. In addition, downregulation of PTEN has been associated with FLS activation in RA patients [28]. In patients, in situ analysis showed increased expression of Bcl-2 in RA-FLS, which correlated with synovial thickening and inflammation [29]. These studies reinforce the important role of Bcl-2 in inhibiting apoptosis in RA-SF, and it is noteworthy that recent studies demonstrated the potential contribution of the apoptotic pathway to the onset of pain pathology in a neuropathic pain model. In a neuropathic pain modeling study by Weng et al[30]. in the spinal cord of unplugged spinal cord nerve ligated rats, the Bax/Bcl-2 ratio and Caspase-3 levels were attenuated reversing the development of mechanical and thermal analgesia.

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