Immune, metabolism and therapeutic targets in RA (Rheumatoid Arthritis)

Keying Liu*

University of Strathclyde, Glasgow, UK

Abstract. Rheumatoid arthritis is a classic autoimmune disease, the pathogenesis of which is closely linked to the auto-reactivity of immune cells and joint inflammation. Three cell types, namely T cells, macrophages and fibroblast-like synoviocytes (FLS), play an important role in the pathogenesis of RA. Numerous studies have pointed to a metabolic reprogramming of T cells, macrophages and FLS in the pathogenesis of RA arthritis, with alterations in different metabolic pathways of cells, mainly producing a shift from oxidative phosphorylation (OXPHOS) to glycolysis, in addition to lipid metabolism and amino acid metabolism which are also altered in the cellular activation state. Metabolic changes are regulated by metabolism-related signalling pathways, and RA is associated with two representative signalling pathways, namely the mTOR signalling pathway and the AMPK signalling pathway. In RA, both signalling pathways are activated or inhibited, and through a series of cascade reactions, different gene expressions are ultimately induced, altering intracellular metabolic pathways and promoting pro-inflammatory functions (e.g. pro-inflammatory cytokine release and FLS phenotypes), or inhibiting the expression of genes related to immune tolerance. Targeting key components of metabolic signalling pathways and key enzymes in cellular metabolic pathways in RA has emerged as a new way of finding drugs for RA, and many modulators targeting these targets have been extensively studied for their therapeutic effects in RA. In this article, we focus on cellular metabolic alterations in RA, related signalling pathways and possible drugs targeting RA metabolic pathways.

Key Words: Immune, metabolism, therapeutic targets, RA(Rheumatoid Arthritis),T cells, macrophages , fibroblast-like synoviocytes (FLS), signalling pathways

1. Introduction

Rheumatoid arthritis (RA) is one of the most common chronic progressive autoimmune diseases today, characterised primarily by inflammation of the synovial membrane of the patient's joints, but should be considered a systemic disease involving extra-articular tissues (e.g. lung, cardiovascular, eyes and skin). One of the major mechanisms responsible for the development of RA is the autoimmune response that exists in the RA patients. The neoantigen is recognized by the major histocompatibility complex molecule (MHC) on T cells and binds to autoantibodies (e.g. rheumatoid factor and anti-citrullinated protein antibodies) produced in response to B cell stimulation. The neoantigen-autoantibody complex in turn induces a variety of immune responses, including complement activation, resulting in autoarticular damage. [1, 2] In RA patients, naive CD4+ T cells are stimulated by inflammatory cytokines to proliferate and differentiate, producing a variety of effector cells, particularly Th17 and Treg, two effector cells that have opposite effects on arthritis in RA: Th17 produces the key pro-inflammatory cytokine IL-17, which can induce the production of osteoclasts, in addition to other pro-inflammatory cytokines such as TNF-α and IL-6 by FLS and macrophages, exacerbating joint inflammation and bone destruction, whereas Treg has immunosuppressive effects and helps to maintain peripheral immune tolerance. [3] Increased number of macrophages at the joint site can be an important indicator of early RA, and as one of the most abundant cells at the site of inflammation in RA. They are also strongly associated with RA pathogenesis. The release of inflammatory cytokines (e.g. TNF-α) and chemokines by macrophages increases the inflammatory response by recruiting more immune cells into the synovial space, promotes the differentiation of naive CD4+ T cells towards pro-inflammatory Th1 and Th17, and activates fibroblasts and osteoclasts, resulting in bone erosion and joint destruction. [4] In RA, FLS is the main resident cell in the synovium and is stimulated by a variety of inflammatory cytokines, which increase its proliferation capacity and express different phenotypes with tissue destructive properties, in addition to causing damage to cartilage tissue, for example, through the secretion of collagenase, matrix protease-1 and other destructive proteases. Besides, FLS increases

* Corresponding author: keying.liu.2019@uni.strath.ac.uk

inflammatory cell infiltration and invasion, causing cartilage degeneration and bone erosion in the joints. [5]
The activation, migration and proliferation of T cells, macrophages and FLS require high levels of energy and nutritional support, both for rapid energy production to meet the increased energy demands of the activated state and for the production of large amounts of biosynthetic intermediates for cell growth and proliferation. This means that when cells transform from a resting state to an activated state, intracellular metabolism undergoes corresponding adaptive changes. Common types of metabolism include oxidative phosphorylation (OXPHOS), glycolysis, the pentose phosphate pathway (PPP), fatty acid oxidation and synthesis, and amino acid metabolism. In the activated state, intracellular glycolysis and PPP are upregulated, producing lactate rapidly and in a hypoxic environment, in addition to the production of ribulose-5-phosphate, an important biosynthetic intermediate, via PPP. Fatty acid metabolism is divided into fatty acid oxidation and fatty acid synthesis, the former being elevated mainly in anti-inflammatory M2 macrophages, Treg and memory T cells, while fatty acid synthesis is more associated with pro-inflammatory immune cells and contributes to various inflammatory responses of cells. In addition, amino acid metabolism also plays an important role in T cells, macrophages and FLS, for example by promoting cell proliferation and nitric oxide release. [6] The reprogramming of metabolic pathways is regulated by multiple signalling pathways. In RA, the mammalian target of rapamycin-regulated protein (mTOR) signalling pathway and AMP-dependent protein kinase (AMPK) signalling pathway are closely associated with metabolic alterations such as upregulation of glycolysis. Modulation of cellular metabolic pathways has emerged as a valuable therapeutic strategy for autoimmune diseases such as RA, and several drug molecules have been shown to be useful in animal models of RA by targeting important components of metabolism-related signalling pathways (e.g. mTOR inhibitor rapamycin, JAK inhibitor tofacitib) and key enzymes in glycolysis and fatty acid metabolism. [7] In this review, we will focus on the metabolic alterations of the three cell types that affect RA (T cells, macrophages and fibroblast-like synoviocytes) in RA, the two metabolic regulatory pathways associated with them, and possible therapeutic targets for cellular metabolism in RA.

2. Cellular Metabolism in RA

2.1 The abnormal metabolism of T cell in RA

Glucose is the main energy sources for human bodies. In contrast to the aerobic oxidation of mitochondria, the glycolytic pathway is a non-oxygen-dependent gluconeogenic pathway that rapidly provides energy and metabolic intermediates. In the absence of antigen activation, T cells convert glucose to pyruvate via the glycolytic pathway, which is oxidized by mitochondria to acetyl coenzyme A. This enters the tricarboxylic acid cycle to produce NADH, FADH2 and GTP, which then undergoes an electron transport system to produce large amounts of ATP to maintain various physiological activities in the resting state of T cells. [8] Self-antigens are recognised by the T cell antigen receptor (TCR) or stimulated by cytokines, and T cells proliferate and differentiate into effector T cells and undergo metabolic reprogramming. [9]
It has been noted that RA patients have altered glucose metabolism after activation compared to healthy patients with activated naive CD4+ T cells, with a low ATP, low intracellular ROS, low lactate, and high NADPH state of energy deprivation. In contrast to activated naive CD4+ T cells from healthy individuals, such T cells from RA patients have impaired glycolysis, as evidenced by glucose shunting into PPP, producing more of the nucleotide synthesis precursors ribose 5-phosphate and NADPH. This is mainly because that, on the one hand, activated naive CD4+ T cells from RA patients have impaired glycolysis of fructose-6-phosphate 2-kinase/fructose-2,6-bis PFKFB3 tends to catalyse the synthesis of fructose-2,6-bisphosphate, an enzyme whose activation enhances glycolysis in cancer cells and is used as a therapeutic target to inhibit cancer cell survival and proliferation. [10] On the other hand, glucose-6-phosphate dehydrogenase (G6PD), a key gatekeeper enzyme for glycolysis into PPP, is overexpressed in RA naïve CD4+ T cells upon activation. G6PD is the catalytic enzyme for the first step of the rate-limiting pathway of PPP, which oxidatively dehydrogenates glucose-6-phosphate to catalyse the production of glucose-6-phosphonolactone, which is then subsequently catalyzed to produce the biologically important glucose-6-phosphonolactone. Yang et al. (2016) observed that naive CD4+ T cells in RA patients had a high level of G6PD/ PFKFB3 and G6PD/ PFKFB3. G6PD/ PFKFB3 ratio was increased and both NADPH and GSH levels affected by PPP were increased, while PFKFB3 levels associated with glycolysis were decreased, demonstrating an impaired glycolytic pathway with enhanced PPP. [11] Furthermore, due to elevated NADPH levels in activated RA, naïve CD4+ T cells exhibit reductive stress. The key kinase activating the G2/M phase block, ataxia telangiectasia mutated (ATM), can not be fully activated in the presence of impaired redox signalling, leading to excessive cell proliferation. [12] In addition, activated naive CD4+ T cells exhibit metabolic abnormalities in RA as evidenced by impaired mitochondrial DNA repair nuclease meiotic recombination homolog 11 (MRE11A) and insufficient expression of n-myristoyltransferase 1 (NMT-1). Impaired MRE11A function allows leakage of mitochondrial DNA into the cytoplasmic matrix, activating caspase-1, which impairs the electron transport chain (ETC) and inhibits ATP production in mitochondria. [13] NMT transfers myristic acid to the N-terminal glycine of the protein, which plays an important role in the activation of adenosine monophosphate-activated protein kinase (AMPK). AMPK senses cellular energy status and inhibits the mammalian target of rapamycin (mTOR1) signalling pathway associated with cellular glucose metabolism, so that deficiency of NMT1 impairs cellular energy perception and results in hyperproliferation and poor differentiation, resulting in

excessive proliferation and poor differentiation, contributing to joint inflammation. [14]

Naïve CD4+ T cells, upon activation, can differentiate into either pro-inflammatory Th17 or Treg, which are associated with inflammation resolution. Th17 and Treg differ significantly in their metabolism. Glycolysis in Th17 is favoured over OXPHOS. High expression of HK2, pyruvate kinase M (PKM), MCT4 and other key enzymes associated with glycolysis, as well as GLUT1, can be observed in the cells. GLUT1, indicating an upregulation of glycolysis in the cells. In addition, Th17 showed a dependence on glutamine catabolism and fatty acid synthesis. Unlike Th17, Treg relies primarily on fatty acid oxidation for energy supply rather than glycolysis or OXPHOS, and Treg is less dependent on amino acids, and reducing extracellular amino acid concentrations can lead to T cell differentiation in favour of Treg over Th1 and Th17.[15] Many studies have pointed to the presence of naive CD4+ T cells in RA patients following activation maldifferentiation, whereby differentiation produces a Th17/Treg imbalance, showing a differentiation preference for Th17, while Treg cell levels are decreased in RA patients. It has been observed that the levels of Th17-related pro-inflammatory cytokines IL-17, IL-6 and TNF-α are significantly increased in the synovium of RA patients compared to healthy individuals, whereas the levels of Treg-related anti-inflammatory cytokines TGF-β and IL-10 are significantly decreased, and the intracellular levels of Foxp3, a key transcription factor regulating Treg differentiation, are also significantly decreased compared to healthy controls. [11, 16] This is consistent with the differentiation tendency of CD4+ T cells in RA. This maldifferentiation is also influenced by cellular metabolism in addition to cytokine-induced transcription factor expression levels. Pyruvate kinase M2 (PKM2) is upregulated in activated CD4+ T cells. PMK2 exhibits kinase activity when tetramerised, catalysing the production of pyruvate from phosphoenolpyruvate in glycolysis. However, PKM2 monomer/dimer, as the main form of PKM2, is less kinase active. The products of reactions catalysed by PKM2 monomer/dimer can be involved in nucleic acid synthesis, which facilitates cell proliferation and inflammation. It has been reported that induction of tetrameric PKM2 will block Th1 and Th17 differentiation. The decrease in ROS levels also has an effect on cell differentiation, leading to the differentiation of CD4+ T cells towards Th1 and Th17.[17] In addition, as a result of glucose shunting to PPP in RA, cellular NADPH levels increase and reactive oxygen species (ROS) depletion occurs. [11]

2.2 The Abnormal Metabolism of Macrophage in RA

There are generally two polarisation outcomes for macrophages: the pro-inflammatory classically activated macrophages (M1-type macrophages) produced in response to M1 stimulation (mainly IFN-γ, lipopolysaccharide LPS, and granulocyte-macrophage colony-stimulating factor GM-CSF), and the anti-inflammatory alternatively activated macrophage (M2-type macrophages) produced in response to M2 stimulation (IL-4, IL-13, IL-10, IL-1, IL-6). In addition to functional differences, there are also metabolic differences between the two types of macrophages: M1 macrophages show a dependence on glycolysis, whereas M2 macrophages are more dependent on OXPHOS for ATP production. The local hypoxic environment in the joint under inflammation induces an upregulation of macrophage glycolysis, leading to a differentiation of macrophages towards M1 macrophages. [18] Studies have shown that 68% of macrophage subtypes in the synovial fluid of RA patients are M1 type, demonstrating a predominance of macrophages that secrete pro-inflammatory cytokines and cause joint inflammation in RA patients. [19] In RA patients, GLUT1 and GLUT3, as well as PKM2, FPKFB3 and HK2, key enzymes involved in glycolysis, were upregulated in macrophages, suggesting that macrophages in RA patients are more likely to be involved in the production of ATP than in healthy individuals. Compared to healthy individuals, macrophages from RA patients can take up more glucose via upregulated glucose transporters for glycolytic capacity, and glycolysis is enhanced in activated macrophages with increased cellular ATP. The enhanced glycolytic pathway means that an excess of its product pyruvate may break the balance between the two processes of TCA cycle catabolism and electron uptake of ETC, allowing pyruvate to accumulate in the mitochondria and ROS to leak from the mitochondria into the cytoplasmic matrix. The leaking ROS will plunge nearby regions into a state of oxidative stress with elevated ROS, affecting the proteins present in them, a typical example being PKM2. ROS oxidative-stimulated PKM2 enters the nucleus and phosphorylates STAT3 transcriptional molecules, rendering them transcriptionally active and promoting the production of large amounts of the pro-inflammatory cytokines IL-1β and IL-6 by macrophages.[9, 20] It was found that phosphorylation of STAT3 and STAT1 proteins in M1-type macrophages was significantly affected by the knockdown of the PKM2 gene, suggesting that oxidative-stimulated dimeric PKM2, in addition to having an effect on the STAT3 signaling pathway, also contributes to pro-inflammatory cytokine secretion and joint destruction through modulation of the STAT1 signaling pathway, worsening the arthritic condition. [21]

Upregulation of key glycolytic enzymes in macrophages also affects macrophage polarization. For example, PKM2 stabilizes HIF-1α, thereby promoting monocyte differentiation towards pro-inflammatory M1-type macrophages, whereas blocking PKM2 entry into the nucleus may favor monocyte differentiation towards M2-type macrophages, which promote inflammation regression. [22] Notably, patients with RA are at higher risk of coronary artery disease (CAD) like atherosclerosis and macrophages from CAD patients share certain characteristics with those from RA. Macrophages from both CAD and RA exhibit elevated cellular ATP and ROS (both mitochondrial ROS and cytosolic ROS) compared to healthy controls, and RA-derived The upregulation of glucose transporters and glycolytic enzymes observed in macrophages of CAD origin was also observed in macrophages of CAD origin, and both CAD and RA

macrophages exhibited higher glucose utilization and secretion of the pro-inflammatory cytokines IL-6 and IL-1β, which could indicate a clinical correlation between CAD and RA. [9, 20]

2.3 The Abnormal Metabolism of FLS in RA

At different stages of RA progression, FLS is activated by different regulatory factors or stimuli from the surrounding hypoxic environment and exhibits aggressive phenotypes that damage the joints and exacerbate joint inflammation. [23] Activated FLS shows varying degrees of alteration in the metabolism of important biomolecules such as sugars, proteins and lipids. Many studies have demonstrated altered glucose metabolism in FLS in RA, and this dysregulation of glucose metabolism is more pronounced in FLS in osteoarthritis (OA) than in OA, as evidenced by an upregulation of glycolysis, PPP and gluconeogenesis. Some studies have observed increased expression of GLUT1 in FLS derived from RA patients compared to FLS in OA. It has been suggested that the upregulation of GLUT1 in RA FLS may be related to its phenotypic and functional transformation, and that FLS with upregulated GLUT1 expression is aggressive and can cause cartilage destruction. Metabolite analysis of diseased synovial fluid compared to normal synovial fluid from RA patients showed a decrease in glucose content, implying that glucose uptake is greatly increased in the heavily proliferating FLS after activation for glycolytic capacity. [24, 25] Furthermore, a study using GC/TOF-MS to analyse the metabolite profile of FLS from RA versus OA showed that various metabolite changes were associated with altered glycolytic pathways in the RA group compared to the OA group, with a significant increase in intermediates of glycolysis and PPP, and that amino acids that can be used as substrates for gluconeogenesis were significantly decreased in RA FLS, possibly due to the fact that glucose entering the glycolytic pathway cannot be the main source of energy and therefore requires the gluconeogenesis pathway to synthesize new glucose to meet energy requirements. [25] Another study found that mRNA expression of mRNA encoding HK2, pyruvate dehydrogenase kinase-1 (PDK1), and mRNA encoding monocarboxylate transporter protein-4 (MCT4), a lactate transporter protein, were increased in RA FLS, again suggesting that FLS glycolysis is upregulated in RA. [24] Furthermore, in contrast to OA FLS, an upregulation of glutaminase 1 (GLS-1) expression, which catalyzes the conversion of glutamine to glutamate, can be observed in RA FLS, while cell proliferation in RA FLS is inhibited upon inhibition of GLS-1, which could indicate the dependence of RA FLS on glutaminolysis. [26]

The shift in pro-inflammatory function of RA FLS is also associated with abnormal lipid metabolism in FLS cells. In RA FLS, choline levels are elevated and the expression of both choline-like transporter proteins CLT1 and CLT2 are upregulated in RA FLS, while inhibition of choline transporters promotes FLS apoptosis, which could indicate enhanced choline uptake in RA FLS. Moreover, the choline pathway was upregulated in RA FLS. Many studies have found that choline kinase (ChoKα), a key enzyme that catalyses the first step of the choline cytidine diphosphate pathway, plays a critical role in the migration and apoptosis of RA FLS. ChoKα plays an important role in the biosynthesis of phosphatidylcholine (PC), which has been shown to be involved in the migration and invasion of cancer cells. Inhibitors of ChoKα have been shown to have some clinical therapeutic value, and inhibition of ChoKα has shown significant improvements in arthritis models. In addition, phospholipase D (PLD) was observed to be associated with pro-inflammatory cytokine and chemokine release from RA FLS, and this enzyme catalyzes the production of choline and phosphatidic acid (PA) from PC, where PA is associated with a number of rapid cellular responses (e.g. cytokine release). [22] Silencing PLD-1 expression in RA FLS and specific inhibition of PLD-1 and PLD-2 both impaired the secretion of cytokines IL-6, IL-8 and chemokine ligand CCL20 in RA FLS, which could indicate an important role of phospholipid metabolism in the expression of pro-inflammatory functions in RA FLS. Other bioactive lipid metabolism is also altered in RA FLS. Sphingosine-1-phosphate (S1P), a lipid involved in the pathogenesis of several autoimmune diseases, is significantly upregulated in RA FLS. In addition, autotaxin (ATX), a lysophospholipase D, was also observed to be upregulated in RA FLS, and inhibition of ATX expression could limit disease pathogenesis in animal models of arthritis. This enzyme catalyzes the conversion of lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA), and high levels of PLC or a low PC/PLC ratio in plasma can be indicative of inflammation. [23]

3. Key Metabolic Pathways in RA

3.1 mTOR signaling pathway

The mammalian target of rapamycin (mTOR) protein can regulate a variety of cellular activities such as autophagy, apoptosis and cellular metabolism to sustain cell growth, proliferation and survival. Increasingly, studies have noted that mTOR plays an important role in the pathogenesis of RA, and inhibitors of mTOR, such as curcumin, sirolimus, etc., can lead to ameliorative disease in murine models of arthritis, not only by reducing cellular infiltration in the joint cavity, but also by reducing the release of pro-inflammatory cytokines and chemokines. [27, 28] In response to inflammation, immune cells activate increased energy demands. mTOR regulates downstream signalling pathways and transcription factors to influence the synthesis or breakdown of biomolecules such as sugars and lipids to accommodate cellular demands for nutrients and ATP. In RA, a transcription factor primarily involved in the mTOR pathway is hypoxia-inducible factor-1α (HIF-1α), which induces upregulation of glucose transporter expression, increasing cellular uptake of glucose, and also increases the expression of glycolysis-related enzymes, such as PFKFB3, contributing to a shift in cellular metabolism from oxidative phosphorylation to the glycolytic pathway. [9, 29] The PI3K/Akt/mTOR pathway is upregulated in RA FLS. mTOR acts downstream of the PI3K/Akt

signalling pathway and the upstream molecule PI3K is activated to phosphorylate PI (4) P and position 3 of PI (4,5) P, generating a ligand for protein kinase B (Akt) and thereby recruiting Akt, which then undergoes a series of processes to activate mTOR with transcription factors HIF-1α, which upregulates glycolytic genes in RA FLS, causes upregulation of RA FLS glycolysis and downregulation of RA FLS cell autophagy resulting in overproliferation and joint destruction. [30] mTOR activation also regulates gluconeogenesis, lipid metabolism and amino acid metabolism in T cells. mTOR activation in activated T cells after TCR stimulation results in the upregulation of key intracellular glycolytic enzymes HK, FPKFB3 and PKM2, resulting in enhanced intracellular glycolysis. In addition, the proliferation of activated T cells is also regulated by mTOR, which facilitates the production of PPP, a biosynthetic intermediate. mTOR upregulation is also associated with T cell differentiation. Upregulation of fatty acid oxidation or inhibition of mTOR leads to enhanced differentiation of Treg cells and facilitates the resolution of inflammation, whereas upregulation of mTOR and thus cellular glycolysis leads to differentiation of T cells towards effector T cells. Inhibition of cytosolic glycolysis or antagonism of mTOR resulted in a decrease in Th1, Th17 differentiation. In addition, HIF-1α is also involved in the mTOR pathway by regulating cellular metabolism and thus the fate of cell differentiation. [31, 32] There are fewer reports related to the effects of mTOR on macrophage metabolism in RA, but it has been suggested that mTOR-related pathways can have effects on glucose metabolism, lipid metabolism and amino acid metabolism in macrophages. Activation of mTOR can regulate glycolysis and lipid synthesis in M1-type macrophages, thereby promoting the release of pro-inflammatory cytokines. For M2 macrophages, activation of the mTOR pathway upregulates glutamate metabolism and regulates cellular utilization of glucose. [33]

3.2 AMPK signaling pathway

AMP-activated protein kinase (AMPK) can sense the ATP/ADP ratio and thus the intracellular energy status by binding directly to adenine nucleotides, which is important for the maintenance of cellular energy homeostasis. Under normal conditions, when intracellular ATP levels are low, AMPK is modified and activated by NMT-1 to alter the metabolic state of the cell by phosphorylating downstream pathways, leading to increased catabolism of sugars, lipids and other biomolecules to fill the energy gap, while inhibiting anabolic reactions such as lipid synthesis and glycogen synthesis to reduce ATP depletion. [34] In RA, T cells have mitochondrial damage due to the absence of the DNA repair nuclease MRE11A, which results in low ATP levels and leakage of ROS into the cytoplasmic matrix. However, AMPK activity is inhibited in RA, mainly due to the inability of AMPK to be modified because of the impaired NMT-1 function, which in turn fails to inhibit mTOR, prompting T cells in RA to continue catabolism such as glycolysis in a state of impaired mitochondria and low ATP levels. This can lead to a state of continuous activation of mTOR and induces T cells to move towards the pro-inflammatory Th1 and Th17, releasing large amounts of pro-inflammatory cytokines and exacerbating joint inflammation in RA. [35] Furthermore, it has been reported that AMPK is associated with a shift in cellular metabolism in macrophage polarization and can regulate the shift from pro-inflammatory M1-type macrophages that utilize glycolysis to M2-type macrophages that utilize OXPHOS and fatty acid oxidation. Although the mechanisms of AMPK regulation of metabolism have not been fully investigated, it has been suggested that AMPK can phosphorylate and thereby inhibit acetyl coenzyme A carboxylase (ACC), which catalyzes the conversion of acetyl coenzyme A to malonyl coenzyme A. Malonyl coenzyme A can inhibit carnitine palmitoyltransferase 1 (CPT1), and given the important role of CPT1 for mitochondrial uptake of fatty acyl coenzyme A, the decrease in malonyl coenzyme A caused by AMPK could promote fatty acid oxidation and inhibit fatty acid production. [36] AMPK has also been associated with altered cellular metabolism in the infiltrative phenotype of RA FLS. It has been suggested that pro-inflammatory cytokine secretion in RA FLS is associated with glycogen synthesis, that knockdown of the gene for the RA FLS glycogen synthesis-related enzyme elevates AMPK, and that treatment of RA FLS with AMPK agonists inhibits the invasion and overproliferation that results from its activation. In turn, knockdown of the alpha subunit of AMPK restores some of the migratory and infiltrative capacity of RA FLS. [37]

4. Abnormal Metabolism as Therapeutic Targets for RA

T cells, macrophages and FLS undergo metabolic reprogramming in RA, key enzymes of the glycolytic process are upregulated or downregulated, and metabolism-related signalling pathways are activated or inhibited, leading to pro-inflammatory cytokine secretion and joint enlargement caused by cellular infiltration. Targeting key enzymes of the metabolic process and key components of the relevant signalling pathways could open up new ideas for the treatment of RA. Indeed, many immunosuppressive agents that are clinically useful in the treatment of RA, such as the mTOR inhibitors rapamycin and sirolimus lipid, the JAK pathway inhibitors tofacitib and ruxolitinib, have been increasingly studied for their modulatory effects on metabolic pathways. In addition, a large number of studies have also indicated that compounds targeting aberrant metabolism in RA have shown clear therapeutic effects in animal arthritis models and in vitro cultured RA cells. [9, 15] Glucose metabolism is dysregulated in RA, and targeting key enzymes in glycolysis is considered to be one of the ideas for developing drugs for RA. 3-Bromopyruvate (BrPA), a specific inhibitor of HK2, a key enzyme in glycolysis, was previously considered primarily as an anticancer agent, but in recent years it has been shown to reduce joint inflammation through inhibition of glycolysis in RA. It was observed that the severity of arthritis was reduced in SKG rats injected with BrPA, and interestingly, BrPA

inhibited the differentiation of Th17 cells cultured in vitro and promoted the differentiation of Treg cells, in addition to inhibiting T cell activation by suppressing activated dendritic cells. This could suggest that BrPA may have a therapeutic effect on RA joint inflammation. [38] In addition, it has been shown that BrPA and another HK inhibitor, 2-deoxyglucose (2-DG), can inhibit pro-inflammatory cytokine secretion from RA FLS cultured in vitro and reduce the arthritis score in a murine model of arthritis, where 2-DG also exhibits an inhibitory effect on PPP. [39]

In addition to molecules that target key enzymes in glycolysis, drugs that target the regulatory inflammatory signalling pathways in RA also have an effect on cellular energy metabolism in RA. Targeting the JAK/STAT pathway has important implications for RA drug development. JAK is activated upon binding of ligands to tyrosine kinase-associated receptors linked to Janus kinase (JAK), which phosphorylates and activates signal transducer and activator of transcription (STAT), which enters the nucleus to regulate transcription of the genes involved, and it has been observed that activation of the JAK/STAT pathway induces upregulation of glycolysis in RA FLS. [30, 40] The JAK inhibitor tofacitab modulates RA FLS mitochondrial function and reduces ROS production, while tofacitab also leads to a decrease in HIF-1α levels and a significant reduction in the level of the glycolysis-related enzyme HK2 in RA FLS. Besides, genes associated with glycolysis, such as GLUT1 and PFKFB3, were also significantly decreased in RA synovial explants after tofacitab treatment. This could indicate that inhibition of the JAK/STAT pathway has an important role in improving both the inflammatory response and cellular metabolism in RA. [41] Metformin can reduce cellular ATP or AMPK kinase through different pathways contributing to AMPK activation, thus inhibiting mTOR and its downstream pathway HIF-1α. mTOR and HIF-1α are both associated with upregulation of cellular glycolysis and glutamine catabolism, and this metabolic alteration is critical for bone erosion and immune cell activation in RA. Many studies have also reported the therapeutic effects of metformin on inflammation, inflammatory cell overproliferation and bone destruction in RA, and in recent years there have been clinical studies demonstrating the positive effects of additional metformin in the treatment of patients with RA. [42]

Increased T-cell fatty acid (FAS) synthesis is associated with excessive proliferation, infiltrative expression and effector T-cell differentiation, and inhibition of FAS synthesis may reduce tissue inflammation and joint cell infiltration. In addition, atorvastatin or 25-hydroxycholesterol inhibit cholesterol synthesis, which results in a decrease in c-Maf/IL-10 expression and is detrimental to the resolution of inflammation. For RA FLS, targeting ChoKα and choline synthesis can inhibit the ability of cells to migrate and promote apoptosis, thereby reducing joint inflammation. [22, 43]

5. Conclusion

In RA, T cells, macrophages and FLS undergo altered cellular metabolism upon activation. providing energy and biosynthetic intermediates for abnormal cell proliferation, differentiation and massive biosynthesis. Glycolysis and PPP are upregulated in cells while lipid metabolism is disturbed. On one hand, the abnormal metabolism will promote the secretion of pro-inflammatory cytokines and generating a variety of cellular activities that contribute to the development of arthritis in RA. On the other hand, anti-inflammatory cells like Treg and M2 macrophages which rely more on fatty acid oxidation are inhibited due to the combined influence of disrupted metabolism and hypoxic environment in the RA joint. Glycolysis was inhibited. In RA, T cell differentiation is shifted towards the pro-inflammatory Th17, which promotes joint inflammation. In addition to T cells, the metabolism of macrophages is also altered. Macrophages in RA are mainly pro-inflammatory M1-type macrophages, which rely mainly on glycolysis for ATP production. Although FLS is not an immune cell, it plays a key role in joint inflammation and bone damage in RA. Dysregulated glucose metabolism, glycolysis, PPP and gluconeogenesis are all upregulated in FLS, which also shows a dependence on glutamine catabolism and lipid metabolism.

In RA, dysregulation of cellular metabolism is regulated by metabolism-related signalling pathways. mTOR is an important regulatory pathway in RA. mTOR can up-regulate cellular glycolysis by progressively activating an important transcription factor, HIF-1α, which enters the nucleus and regulates gene expression, promoting the release of pro-inflammatory cytokines and the expression of glycolysis-related enzymes. In addition, mTOR regulates glycolysis together with upstream PI3K/Akt and causes cellular hyperproliferation. mTOR is inhibited by AMPK, but AMPK is inhibited in RA and its activation has an ameliorative effect on RA arthritis. the JAK/STAT pathway is also involved in the upregulation of expression of key enzymes in glycolysis and has an important impact on RA pathogenesis. Targeting dysregulated metabolic processes and their regulatory pathways has therapeutic effects in RA. Inhibition of key glycolytic enzymes (e.g. BrPA and 2-DG) and upregulation of key glycolytic pathways (e.g. AMPK agonist metformin and JAK inhibitor tofacitib) have been shown to have therapeutic effects in clinical and numerous in vitro and animal studies. Although there are few studies on the metabolism of immune cells, more research is needed on the specific alterations on lipid and amino acid metabolism of key cells in RA, and few researches are carried out on the effects of metabolic signalling pathways in different cells in RA. In addition, the impact of metabolic regulatory pathways on different cellular metabolism in RA is still poorly studied, within which potentials of new therapeutic targets on metabolisms in RA may be discovered.

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