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REVIEW

New Targets for Immune Inflammatory Response in Rheumatoid Arthritis: Focus on the Potential Significance of N6-Methyladenosine, Ferroptosis and Cuproptosis

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Abstract: Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovitis. It has a high prevalence worldwide, significantly impacting patients ' quality of life. There are still numerous obstacles and problems in the treatment of this disease. In the RA patients and RA animal models, the inflammatory response mainly involves abnormal activation of immune cells, such as T cells and macrophages. These cells release pro-inflammatory cytokines and trigger autoimmune reactions, ultimately causing irreversible joint tissue damage. The pathogenesis of RA is complex, involving genetic and environmental factors. Genetic factors increase the risk of disease, while environmental factors, such as infection and smoking, can trigger the onset of disease. An in-depth study of its pathogenesis and new therapeutic targets is of great significance in improving the therapeutic effect of RA. Recently, m6A methylation, an RNA modification method, has played an important role in regulating gene expression and disease progression. This modification significantly regulates immune inflammatory responses in RA, providing new insights for potential therapeutic approaches. Moreover, ferroptosis and cuproptosis, two new forms of cell death, have gradually been recognized to play an important role in the pathogenesis of RA. Ferroptosis is characterized by an imbalance in intracellular iron homeostasis and the production of reactive oxygen species, while cuproptosis involves the accumulation and metabolic abnormalities of intracellular copper. These processes play a key role in the immune inflammatory response of RA and have become potential therapeutic targets. The current review discusses the research progress of m6A methylation, ferroptosis, and cuproptosis in the pathogenesis of RA and elucidates their interactions. An in-depth understanding of these new targets might provide new strategies and drug design ideas for the treatment of RA, thereby improving the prognosis and quality of life of RA patients.

Keywords: rheumatoid arthritis, immune inflammation, n6-methyladenosine, ferroptosis, cuproptosis

Introduction

Rheumatoid arthritis (RA), a chronic autoimmune disease,[1] is characterized by joint destruction and swelling, pain, and morning stiffness.[2] RA follows a chronic, relapsing-remitting course, frequently resulting in joint deformity, functional impairment, and progressive loss of mobility.[3] The disease affects up to 1% of the population, with a higher prevalence in women compared to men.[1,4] The hand, foot, and knee joints are the most affected parts.[5] Beyond the joints, RA can also impact blood vessels, kidneys, heart, lungs, and liver,[6] leading to extra-articular manifestations, such as rheumatoid nodules, Sjogren's syndrome, and pulmonary fibrosis.[7] Despite advancements in understanding the pathogenesis of RA, the disease's heterogeneity continues to limit therapeutic efficacy, necessitating further research and exploration.[8]

Recently, epigenetics has significantly contributed to arthritis research, particularly in studying RNA modification.[9] While DNA and histone modifications are known to be associated with RA, there are few studies on the role of m6A modification in RA.[10] The m6A modification involves complex enzymatic processes.[11,12] These enzymes play a crucial role in cellular activities[13] and regulate diverse functions at the RNA level.[14] Studies indicated that m6A modification is

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vital for biological processes, such as immune response,[15] and its dysregulation is closely related to human diseases, including RA.[16] To improve the quality of life of RA patients, it is essential to explore the role of m6A methylation in RA and identify new therapeutic targets, which is a current research hotspot. Ferroptosis and cuproptosis, forms of programmed cell death, are critical for maintaining homeostasis.[17–19] Research showed that ferroptosis is associated with joint degeneration, and repairing the lipid peroxide can improve this process.[20–22] The accumulation of Fe2+ in chondrocytes of RA patients triggers oxidative stress, promoting inflammation and joint destruction.[23] Copper, as an enzyme cofactor, helps maintain intracellular copper stability.[24–26] Cuproptosis is related to mitochondrial respiration and involves lipid acylation, protein aggregation, and the loss of iron-sulfur cluster proteins, leading to cell death.[27] Cuproptosis may aggravate immune cell death and joint tissue damage by damaging mitochondrial structure and function and promoting oxidative stress. Copper overload in RA synovial fluid disrupts mitochondrial respiration by binding to lipoylated TCA cycle proteins (eg, DLAT), leading to toxic aggregation and energy crisis.[27] It has become a new therapeutic target for RA and provides a new idea for RA treatment.[28]

In this paper, Web of science, PubMed, Elsevier, Sci-hub are selected as the data source. At the same time, in order to ensure the comprehensive and accurate retrieval of data, the index is selected as SCI-EXPANDED / SSCI. This paper finally determines the new target of RA: m6A methylation retrieval strategy is TS = (‘ N6-methyladenosine ‘OR ‘m6A ‘), ferroptosis retrieval strategy is TS = (‘ Ferroptosis ‘OR ‘iron death ‘). The search strategy of cuproptosis is TS = (‘Cuproptosis ‘ OR ‘ Copper death ‘), t the period time is from January 1994 to June 2024, and the search deadline is June 15,2024. The literature types are Articles and Review Articles. A total of 5868 papers on m6A methylation, 9983 papers on iron death and 895 papers on cuproptosis were obtained.

The current review summarizes the existing evidence on the interplay between m6A methylation modification and cuproptosis and ferroptosis pathways. This review also highlights the potentials of m6A, cuproptosis, and ferroptosis to guide clinical applications and explore their underlying potential pathological mechanisms.

N6-Methyladenosine

N6-Methyladenosine (m6A) and Its Regulatory Factors

The m6A, one of the most common chemical modifications in eukaryotic mRNAs, is the methylation of the sixth nitrogen position of the adenosine base of mRNA.[29] The methylation at m6A involves three types of regulatory factors, including writer, eraser, and reader. The writer catalyzes the methylation of RNA and maintains its stability.[12] Erasers remove methyl groups from RNA, mediating the demethylation process.[11] The reader triggers a series of downstream biological reactions by recognizing m6A.[30] These three types of regulatory factors perform distinct functions but work cooperatively to complete the m6A methylation process[31] (Figure 1). The m6A methyltransferases include METTL3 (Methyltransferase like 3), METTL14(Methyltransferase like 14), and WTAP (Wilms tumor 1-associated protein).[32] METTL3 and METTL14, as core members, catalyze the m6A methylation process of mRNA by forming stable heterodimers.[33] Although WTAP itself does not have methyltransferase activity, it is involved in biological processes such as cell cycle regulation, cell proliferation and apoptosis by interacting with other factors, and indirectly affects the global level of m6A modification.[13,34] On the other hand, demethylases such as FTO (Fat mass- and obesity-associated gene) and ALKBH5(Human AlkB homolog 5) play an indispensable role in the reversible regulation of m6A modification. FTO and ALKBH5 are responsible for demethylation.[35] FTO affects gene expression regulation through its demethylation activity, revealing the complexity of m6A modification in post-transcriptional regulation and its association with gene expression regulation. ALKBH5 further expands the functional category of m6A modification, and shows the extensive influence of m6A modification in RNA life cycle by regulating RNA metabolism, assembly, nuclear export and other processes.[36] Reading proteins include YTH (YT521-B homology) domain proteins, nuclear heterogeneous ribonucleoproteins, and eukaryotic initiation factor (eIF), which are mainly responsible for promoting RNA translation, degradation, stabilization, splicing, processing, and maturation.[14] YTHDC1(YT521-B homology-domain-containing protein 1), the only YTH family methyl-binding protein located in the nucleus, affects the nuclear localization of RNA.[37]

Keywords condense the core and essence of a paper. Through keyword co-occurrence analysis, research hotspots in a subject field can be found. VOSviewer was used to draw a keyword co-occurrence network view of 5868 articles, and

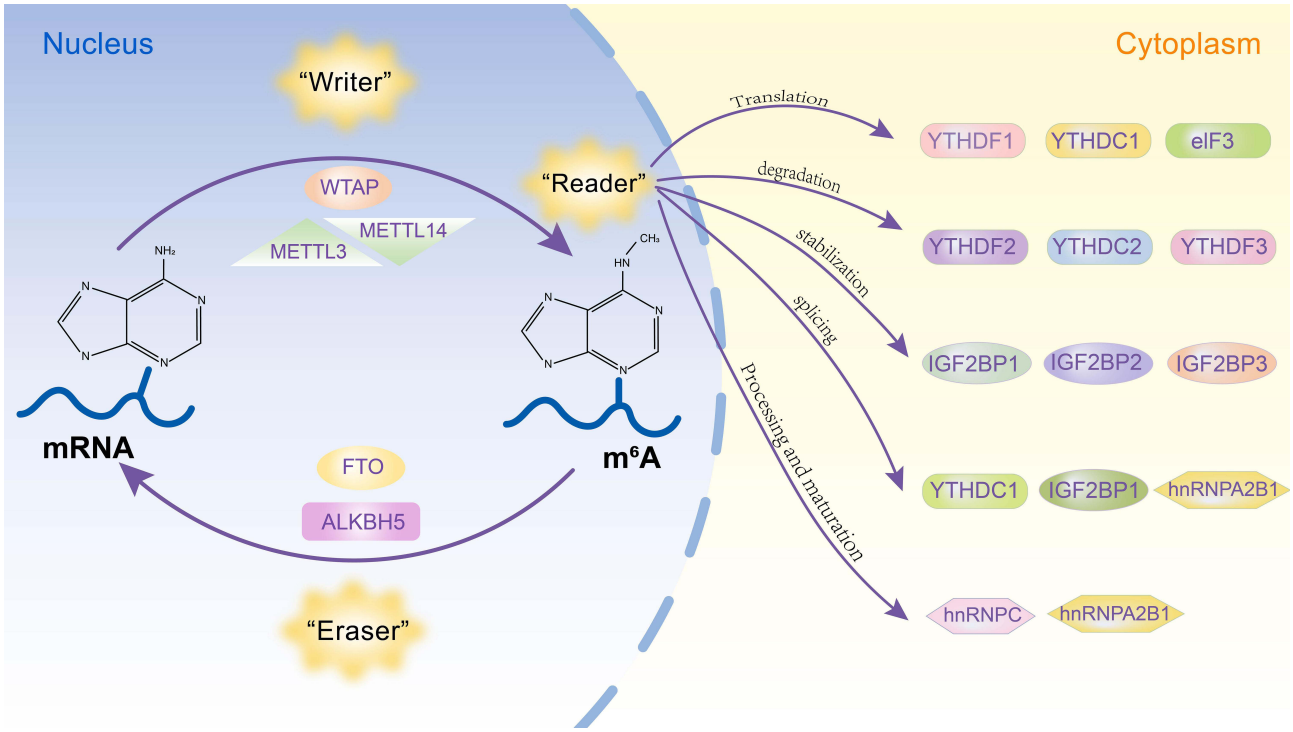

Figure 1 Methylation of m6A RNA and its molecular mechanism. In order to add, remove, or recognize m6A, m6A RNA is methylated by its writer, eraser, and reader, respectively. All aspects of RNA metabolism, such as mRNA translation, RNA degradation, stability, splicing, processing and maturation, are affected by m6A methylation. **Abbreviations**: WTAP, Wilms Tumor 1 Associated Protein; METTL3, methyltransferase like 3; METTL14, Methyltransferase-Like Protein 14; FTO, fat mass and obesity-associated protein; ALKBH5, Alk B homologue 5; YTHDF, YTHDF protein family; YTHDC, YTHDC protein family; eIF3, E74 like ETS transcription factor 3; IGFBP1-6, Insulin-like growth factors binding protein1-6; hnRPA2B1, Recombinant Heterogeneous Nuclear Ribonucleoprotein A2/B1; hnRPC, heterogeneous nuclear ribonucleoprotein C Gene.

30 key keywords with a frequency greater than or equal to 5 were selected for visualization. The results are shown in Figure 2. The larger the circle node is, the more the keywords appear, and the more it can represent the hot spots in the field. The node connection represents the correlation strength, and the coarse connection indicates that the more times the two appear in the same literature; node colors represent different clusters, that is, research topic.

The Role of m6A in RA Immune Inflammatory Response

RA is characterized by immune cell infiltration, excessive proliferation of fibroblast-like synovial cells, and destruction of cartilage and bone. The core pathological mechanism involves macrophage-like synovial cells producing pro-inflammatory cytokines, including IL-1(Interleukin-1), IL-6(Interleukin-6), and TNF (Tumor Necrosis Factor).[38] Several studies demonstrated that regulating immune cells is a critical target for RA treatment. The m6A methylation, an important regulator of gene expression, is involved in various biological processes. Research indicated that m6A methylation modification also plays a role in the pathogenesis of RA. It has been found that m6A methylation modification is also involved in the pathogenesis of RA. High-throughput sequencing combined with methylated RNA immunoprecipitation (MeRIP-seq) and RNA sequencing were used to evaluate the whole transcriptome m6A modification in the synovium of RA patients, revealing the relationship between m6A modification and RA synovial inflammation.[39,40] Therefore, regulating immune cells through epigenetic modification is expected to provide a new method for the treatment of RA.

Several studies showed that reading protein IGF2BPs (Insulin-like growth factor 2 mRNA-binding proteins) could regulate the polarization of M1 macrophage and inflammation markers during the progression of RA.[33] Moreover, research also indicated that METTL14 knockdown could down-regulate the expression levels of m6A in RA patients, promoting the secretion of two inflammatory factors IL-6 and IL-17.[41] It was found that the YTHDF2 mRNA expression levels in RA patients were significantly lower compared to healthy controls and were negatively correlated with ESR

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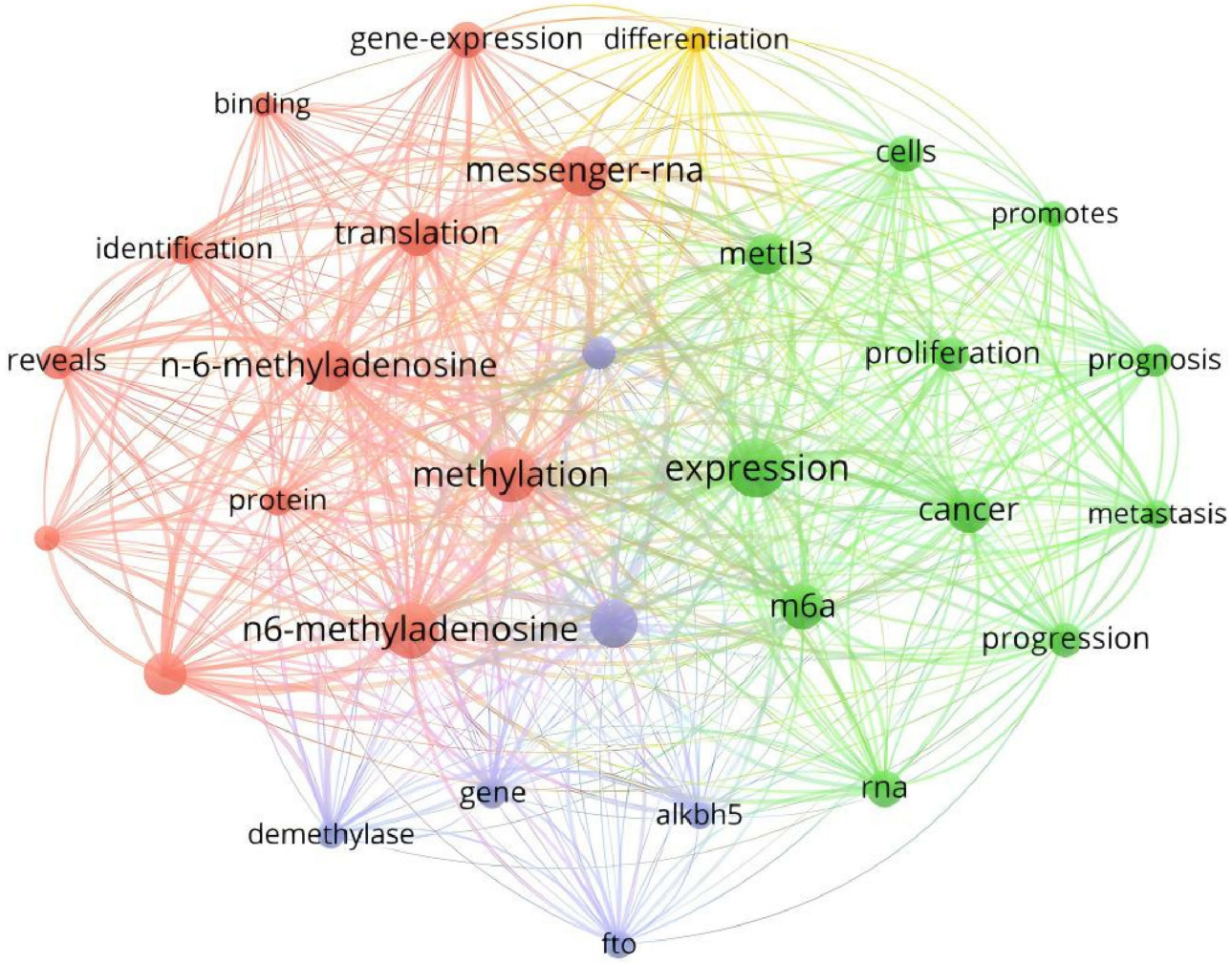

Figure 2 Keywords co-occurrence of m6A methylation.

(Erythrocyte sedimentation Rate), CRP (C-reactive protein) level, WBC (White blood cell) count, neutrophil count, percentage, and NLR value. This suggested that YTHDF2 played a regulatory role in the mechanisms underlying RA, and m6A readers might represent potential targets for regulating the inflammatory response in RA.[42]

There is an imbalance of inflammatory polarization in RA, characterized by a sharp increase in inflammatory markers and heightened inflammatory response. WTAP might be involved in the methylation process of ETS1(ETS proto-oncogene 1). After WTAP intervention, the methylation level of ETS1 m6A changed. The overexpression or interference of circ _ 0066715 reduced or increased WTAP expression levels.[43] In RA, m6A modification induced by ALKBH5 inhibited NLRP3(NOD-like receptor thermal protein domain associated protein 3) mRNA level through YTHDC2(YT521-B homology-domain-containing protein 2), with NLRP3 being a crucial factor in RA progression. The NLRP3 level increased significantly in synovial tissues and fibroblast-like synoviocytes (FLS) of RA patients. ALKBH5 could bind to NLRP3, and inhibiting the ALKBH5 reduced FLS proliferation and inflammatory factor levels. Conversely, the overexpression of NLRP3 neutralized the effects of ALKBH5 in FLS[44] (Table 1). Recent studies further delineate the dual roles of m6A regulators in RA pathogenesis. For instance, ALKBH5-mediated demethylation of NLRP3 mRNA enhances its stability, thereby promoting inflammasome activation in synovial fibroblasts.[45] Conversely, METTL3 overexpression stabilizes TNF-α transcripts through m6A modification, amplifying NF-κB signaling and synovial inflammation.[46] Additionally, METTL14-mediated m6A modification of TNFAIP3 (a key suppressor of NF-κB) is significantly downregulated in RA PBMCs, leading to unchecked inflammatory

Table 1 Regulatory Factors Associated with m6A Methylation in RA

Function	Regulatory Molecules	Sample Source	Expression Trend	Regulation	Reference
Writer	METTL3	PBMC	↑	LPS reduces the inflammatory response of macrophages through the NF-κB signaling pathway.	[47]
Writer	METTL3	FLS	↑	It promotes FLS activation and inflammatory response through NF-κB signaling pathway.	[46]
Writer	RIPK2	MH7A cells	↑	LPS induces p38 and IκB-α signaling.	[39]
Writer	WTAP	MH7A cells	↑	MTTL3-MTTL14-WTAP complex was formed by binding MTTL3.	[39]
Writer	JAK3	MH7A cells	↑	Mediates and participates in the formation of cytokines.	[39]
Writer	TNFRSF10A	MH7A cells	↓	Activation of NF-κB pathway promotes cell proliferation and migration.	[39]
Reader	IGF2BPs	Synovial tissues	↑	Regulate G2 / M transition and affect the polarization of M1 macrophages.	[33]
Reader	IGFBP2	FLS	↑	Regulation of neuropeptides GHR and NPR 2 affects the interaction between FLS.	[48]
Reader	SMOC2	Synovial tissues	↑	The expression of MYO1C was controlled by m6A modification mediated by ALKBH 5.	[49]
Eraser	FTO	Peripheral blood	↓	The expression of MYO1C was controlled by m6A modification mediated by ALKBH 5.	[42]
Eraser	ALKBH	Peripheral blood	↓	Mediated m6A methylation reversal.	[42]

Abbreviations: RIPK2, receptor Interacting Serine/Threonine Kinase 2; JAK3, Janus kinase-3; TNFRSF10A, tumor necrosis factor receptor superfamily member 10A; IGF2BP3, insulin-like growth factor 2 mRNA-binding protein 3; IGFBP2, insulin-like growth factor binding protein 2; SMOC2, secreted modular calcium-binding protein 2.

responses.[41] These findings underscore the therapeutic potential of targeting m6A machinery to restore immune homeostasis in RA.

In human RA synovial tissues and rat AIA model, the expression levels of METTL3 were significantly up-regulated. METTL3 knockdown inhibited the levels of IL-6, matrix metalloproteinase (MMP)-3, and MMP-9 in human RA-FLSs and rat AIA FLSs. However, METTL3 overexpression increased these levels. Moreover, in FLSs, METTL3 could activate the nuclear factor NF-κB signaling pathway. This indicated that METTL3 might promote FLS activation and the inflammatory response through NF-κB signaling pathway.[39,50]

Ferroptosis

Mechanism of Ferroptosis

Ferroptosis, first proposed by Dixon et al in 2012, is a novel form of programmed cell death[51] caused by the accumulation of iron-dependent lipid peroxides.[52–54] The primary mechanism of ferroptosis is related to iron metabolism disorders, imbalances in the system XC-system, and the accumulation of lipid peroxides (Figure 3). First of all, iron metabolism disorders play a key role. When intracellular iron homeostasis is out of balance, especially when the level of free iron is abnormally elevated, they can catalyze fatty acid peroxidation and promote the formation of toxic lipid peroxides. Secondly, the imbalance of the system XC-system is also crucial. The system consists of two subunits, SLC7A11 and SLC3A2, which are responsible for transporting cystine into cells and converting it into cysteine, which is an essential precursor for the synthesis of antioxidant glutathione (GSH). Once the system XC-function is impaired, GSH synthesis is blocked, and the ability of cells to resist lipid peroxidation is weakened, which in turn aggravates the occurrence of ferroptosis. Finally, the accumulation of lipid peroxides is the direct cause of ferroptosis. These highly active molecules can destroy the cell membrane and organelle membrane structure, causing a complete collapse of cell function. In summary, the mechanism of ferroptosis involves the interaction of iron metabolism, system XC-system function and lipid peroxidation, which jointly regulate this unique cell death process.

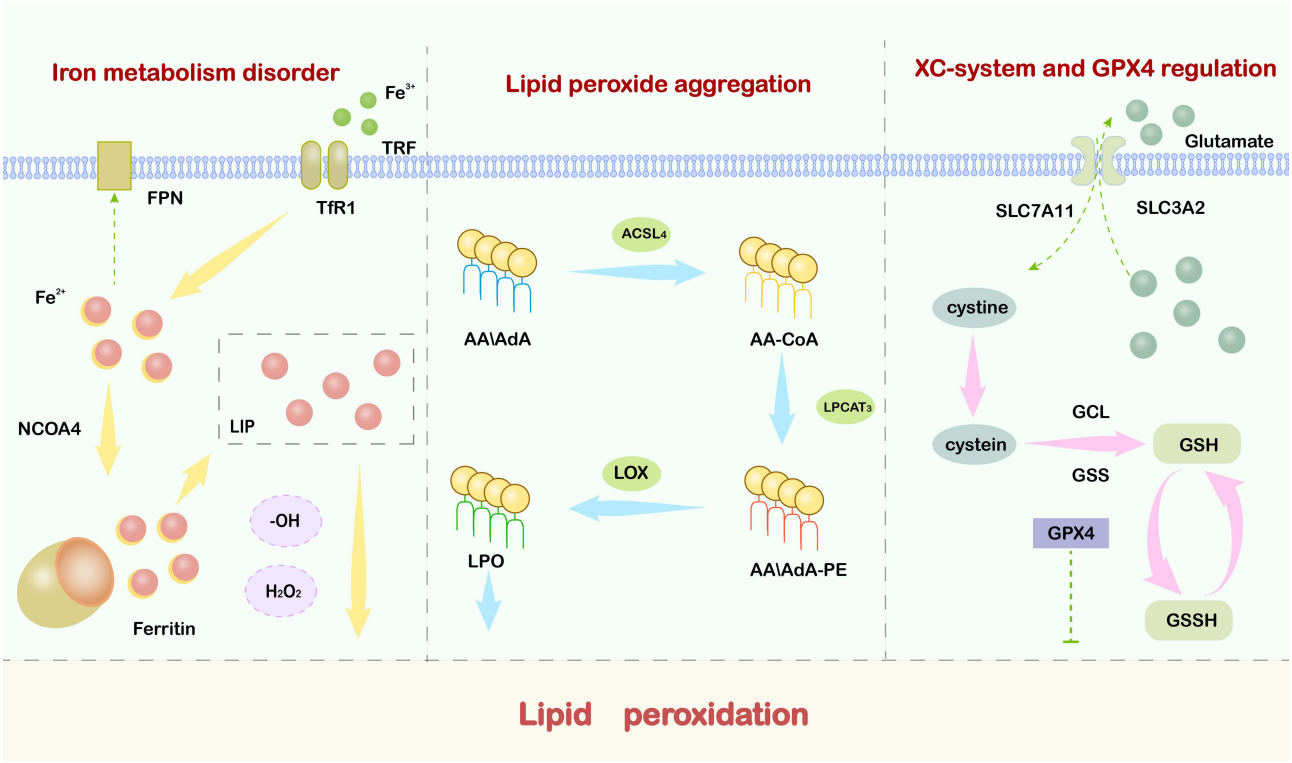

Figure 3 Signal transduction and mechanism of ferroptosis.
Abbreviations: TRF, transferrin; TFR1, Transferrin receptor protein 1; NCOA4, Nuclear Receptor Coactivator 4; LIP, labile iron pool; SLC7A11, Solute Carrier Family 7 Member 11; SLC3A2, Recombinant Solute Carrier Family 3, Member 2; GSH, Glutathione; GPX4, Glutathione peroxidase 4; GSSG, Oxidized glutathione; ACSL4, Human Acyl Coenzyme A Synthetase Long Chain Family, Member 4; LPCAT3, Human Lysophosphatidylcholine Acyltransferase 3; LOX, Lipoxygenase; AA, arachidonic acid; AdA, adrenic acid; LPO, Lipid peroxidation.

Iron Metabolism Disorder

Iron metabolism involves the transport, distribution, storage, utilization, transformation, and excretion of iron within organisms after absorption.[55] Iron ions usually bind with transferrin (TRF) as trivalent iron and enter cells through TRF. Once inside, trivalent iron is reduced to divalent iron by metal reductase, forming various iron-binding complexes that perform multiple physiological roles.[56] When these complexes become saturated, the excess divalent irons accumulate in the cell, creating an unstable iron pool (labile iron pool, LIP). The iron ions in this iron pool are highly active and easy to participate in the free radical formation reaction, especially the reaction with hydrogen peroxide to form hydroxyl radicals. This process is called the Fenton reaction. Hydroxyl radical is a highly destructive oxidant, which can cause peroxidation damage of lipids, proteins and DNA, eventually leading to the destruction of cell structure and function and inducing ferroptosis.[57] In order to maintain the balance of intracellular iron ions and prevent the occurrence of ferroptosis, cells have evolved a set of precise regulatory mechanisms. Among them, ferroportin (FPN) plays a crucial role. FPN is the only protein found so far that can transport intracellular iron ions out of the cell in an inverse concentration gradient, and its activity is strictly regulated to ensure that excess iron can be discharged out of the cell in time when needed. This process not only reduces the concentration of intracellular free iron ions, but also inhibits the occurrence of Fenton reaction and reduces the oxidative stress burden of cells, thus effectively inhibiting the occurrence of ferroptosis.[58] Moreover, the increased expression levels of the intracellular iron stress response protein prominin2 can also transport excess iron out of cells by promoting the formation of iron-containing exosomes and multivesicular bodies.[59] Therefore, the transport of iron ions from the cell to the extracellular environment is crucial for maintaining cellular iron balance.

XC-System and GPX4 Regulation

Under physiological conditions, the XC-system is composed of the solute carrier transport family members SLC7A11 and SLC3A2. This system transports cystine into the cell where it is reduced to cysteine for the synthesis of the primary

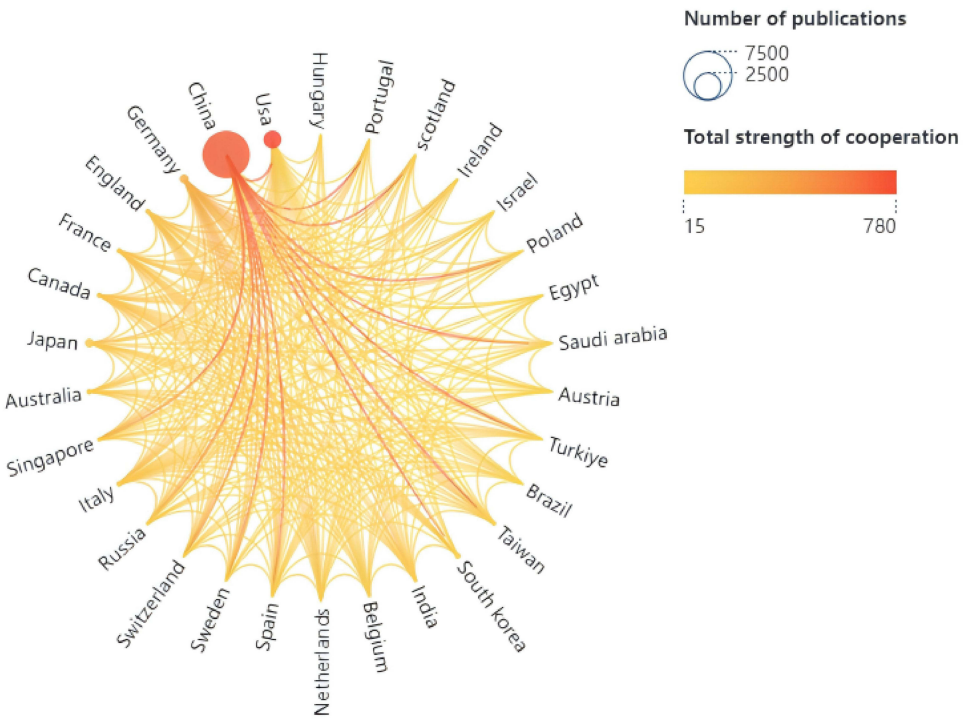

Figure 4 Ferroptosis release country and cooperation index chart.

intracellular antioxidant glutathione (GSH).[60] Recombinant glutathione peroxidase 4 (GPX4) is a selenoprotein that not only effectively reduces peroxides but also inhibits the activation of arachidonic acid (AA) metabolic enzymes during phospholipid peroxidation, thereby inhibiting ferroptosis.[61] GSH serves as an essential cofactor for GPX4, enabling the conversion of the reduced GSH into oxidized GSH (GSSG) and the reduction of lipid peroxides, mitigating oxidative stress damage.[62] SLC7A11 is the principal subunit of the XC-system, functioning as a cystine transporter that facilitates the exchange of cystine and glutamate in cells, thereby promoting GSH synthesis and inhibiting ferroptosis.[63] Consequently, the XC-system and GPX4 are essential regulatory targets in the amino acid metabolism involved in ferroptosis.

Lipid Peroxide Aggregation

The accumulation of lipid peroxides is central to ferroptosis. Polyunsaturated fatty acids, such as AA or adrenic acid (AdA), are particularly susceptible to oxidative reactions during ferroptosis. AA or AdA is catalyzed by three acyl-CoA synthases long-chain family 4 (ACSL4) to AA-CoA and AdA-CoA and then esterified by lysophosphatidylcholine acyltransferase 3 (LPCAT3) to phosphatidylethanolamine (PE) to form AA-PE and AdA-PE, which are finally oxidized by lipoxygenase (LOX) to lipid peroxidation (LPO).[21,22,64,65]

A total of 9983 literatures were analyzed by VOSviewer and Scimago Graphica. In Figure 4, the top 30 countries with the most research on ferroptosis were screened out in the map of publishing countries and cooperation index. The size of the network node represents the number of documents published by the country. The larger the node is, the more the total number of documents published by the country is. The deeper the color of the connection between the nodes, the closer the cooperation between the two countries.

The Role of Ferroptosis in RA Immune Inflammatory Response

Iron Metabolism Disorder in RA

The imbalance of iron metabolism is a key factor in the development of RA, aggravating the disease through various mechanisms, such as enhancing oxidative stress, triggering an inflammatory response, and impairing immune cell function.[66] In RA patients, the indicators of iron metabolism, such as serum iron and ferritin, often show an increasing

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trend.[67] Notably, iron concentrations are positively correlated with the severity of joint inflammation, a relationship confirmed by several studies.[68] Therefore, a deeper understanding of the relationship between iron metabolism and RA is crucial for developing new therapeutic strategies. RA patients typically exhibit elevated iron metabolism indicators, with excessive iron in synovial fluid closely linked to the disease severity, as reflected in the lipid peroxidation of focal monocytes/macrophages. Studies showed that anti-inflammatory macrophage M2 was highly sensitive to iron-induced ferroptosis, while pro-inflammatory macrophage M1 was less affected. Moreover, p62/SQSTM1(sequestosome 1)-dependent degradation of GPX4 in both cell types plays a significant role in this process. Furthermore, animal experiments demonstrated that the ferroptosis inhibitor liproxstatin-1 could alleviate the progression of K/BxN serum transfer-induced arthritis (STIA) in mice and increase the proportion of M2 macrophages.[69] Farther, the ferroptosis inhibitor liproxstatin-1started at the presymptomatic stage in collagen-induced arthritis (CIA) model mice, and GPX4 overexpression in M2 macrophages at the onset of collagen antibody-induced arthritis (CAIA) protected M2 macrophages from ferroptotic cell death and significantly prevented the development of joint inflammation and destruction.[70]

GPX4 Regulation and RA

The abnormal activation of synovial fibroblasts (SFs) plays a crucial role in RA. Semaphorin 5A levels were significantly elevated in synovial fluid and synovial tissue of RA patients, primarily derived from CD68+ synovial macrophages. This increase led to enhanced binding between Semaphorin 5A and its receptor, promoting cytokine secretion, proliferation, migration, and reduced apoptosis. Semaphorin 5A can significantly eliminate the ferroptosis activator RSL3-induced SFs mitochondrial reduction, increase membrane density, and ridge rupture. It enhances GPX4 expression and SREBP1/SCD-1 signaling pathway by activating the PI3K/AKT/mTOR signaling pathway, thereby inhibiting ferroptosis in the SFs of RA patients.[70] Importantly, clinical studies reveal that GPX4 downregulation in RA synovial tissue inversely correlates with elevated inflammatory markers (eg, CRP and ESR), underscoring its role as a critical anti-ferroptotic regulator linked to disease severity. Research indicated that glycine might promote ferroptosis in RA by increasing the expression levels of SAM-related GPX4 and FTH1, as well as promoting the methylation of GPX4 promoter, leading to reduced GPX4 and enhanced ferroptosis in RA FLS. GPX4, an anti-ferroptosis marker, is inactivated by the ferroptosis activator RSL3, which binds to GPX4, mediating GPX4-regulated ferroptosis.[71] Moreover, the ferroptosis-related gene *SLC2A3* has been identified as a potential biomarker for RA diagnosis and treatment. The treatment of RA FLS with RSL3 could down-regulate SLC2A3 expression, inducing ferroptosis in RA FLS.[72]

Lipid Peroxide Aggregation of RA

The accumulation of lipid peroxides is central to the process of ferroptosis. This process is highly relevant in the context of RA, where oxidative stress acts as the key pathogenic marker and exacerbates the local microenvironment of RA lesions. The oxidative stress in RA not only enhances the proliferation of abnormal synovial cells but also aggravates the inflammatory inflammation, leading to greater joint damage.[73] Iron overload in RA synovium drives Fenton reactions, generating hydroxyl radicals that exacerbate lipid peroxidation and synovial inflammation. Notably, SLC7A11 (a cystine/glutamate antiporter) exhibits stage-specific roles in RA: its compensatory upregulation in early-stage RA fibroblast-like synoviocytes (FLS) counteracts ferroptosis by maintaining glutathione synthesis, while insufficient SLC7A11 activity in advanced stages may exacerbate lipid peroxidation and disease severity.[23] Emerging evidence implicates the STING pathway in ferroptosis-driven inflammation. In RA synovium, mitochondrial DNA leakage activates STING, which upregulates ACSL4, amplifying lipid peroxidation and macrophage ferroptosis. This creates a feedforward loop of inflammation and tissue damage.[74] Additionally, the retinoic acid pathway modulates ferroptosis sensitivity; silica-induced ferroptosis in dendritic cells triggers retinoic acid signaling, exacerbating fibrosis and synovial inflammation —a mechanism potentially relevant to RA progression.[75] Galectin-1, a bioactive peptide, has been reported to exhibit anti-inflammatory and anti-proliferative effects in RA FLS. It operates through the p53/SLC7A11 axis to promote the accumulation of lipid peroxides and iron deposition, significantly enhancing TNF-α-induced ferroptosis in RA cell models.[76] In a therapeutic context, combined treatment strategies involving drugs and hydrotherapy have shown promising potential in reducing oxidative damage to proteins, lipids, and DNA by increasing the activity of antioxidant enzymes, such as SOD and GPX. Moderate-intensity exercise, including hydrotherapy, has been found to improve the

oxidative-antioxidative balance in RA patients, thereby reducing the disease severity.[77] Moreover, in pediatric cases, the activation of lipid peroxidation and the impairment of antioxidant defenses have been implicated in sustaining high levels of immune inflammation in conditions like juvenile idiopathic arthritis (JIA) and juvenile scleroderma (JS).[78] This highlights the crucial role of oxidative stress and lipid peroxidation in the pathology of these diseases (Table 2).

Significance of Ferroptosis in the Clinical Treatment of RA

Iron plays an important role in the clinical manifestations of RA. As a key trace element, iron deficiency may aggravate the symptoms of fatigue and fatigue in RA patients, affecting the disease process and quality of life.[86] In response to this phenomenon, potential therapeutic strategies include direct iron supplementation to correct iron deficiency, and the use of iron chelators and antioxidants to indirectly improve the metabolic environment of iron. Iron chelators such as leflunomide and desferrioxamine (DFO), have shown potential in RA management. While leflunomide primarily inhibits immune hyperactivation, its metal-chelating properties may indirectly modulate iron metabolism. In contrast, DFO directly targets iron overload and oxidative stress in RA joints. Unlike leflunomide, which acts indirectly, DFO inhibits the Fenton reaction by chelating free iron, thereby reducing hydroxyl radical (·OH) generation, synovial lipid peroxidation, and ferroptosis.[87,88] Animal studies demonstrate that DFO treatment significantly upregulates immunomodulatory genes (Tgfb, Hgf, and Tsg-6) in mesenchymal stem cells and enhances anti-inflammatory effects.[89] A clinical study further indicates that DFO improves chronic disease anemia (CDA), reduces IL-6 levels, and restores joint function in RA patients.[90] Although its main mechanism of action is to inhibit the overreaction of the immune system, thereby reducing joint inflammation and damage, but its metal chelating properties may also affect the metabolism of iron to a certain extent. Iguratimod tablets combine with copper, zinc and other metal ions to form complexes, which in turn affect the activity of some enzymes and the release of cytokines, and indirectly improve the utilization of iron.[91] However, it should be noted that leflunomide is not a direct treatment for iron, and its efficacy may be affected by many factors. Antioxidants such as anthocyanins in berries and vitamin C in citrus fruits have anti-inflammatory properties and can improve the symptoms of RA.[92]

Recent studies reveal that ferroptotic cell death releases damage-associated molecular patterns (DAMPs), including HMGB1, which activate NF-κB signaling in neighboring macrophages. This perpetuates IFN-I production, creating a pro-inflammatory milieu that exacerbates joint erosion.[93] Furthermore, Drosophila models demonstrate that ferroptosis-like death amplifies chronic inflammation via lipid peroxide-mediated activation of the IMD pathway, providing

Table 2 The Effect of Ferroptosis-Related Genes on RA

Ferroptosis Gene	Cells that have an Impact in RA	Function	Reference
GPX4	Polymorphonuclear leukocytes, synovial cells	Inhibition of lipid peroxidation, negative regulation of ferroptosis	[71]
ACSL4	Synovial fibroblasts	Regulation of fatty acid metabolism, positive regulation of ferroptosis	[71]
SLC7A11	Synovial cell	Regulation of glutamine metabolism, negative regulation of ferroptosis	[72]
TFR1	Macrophages, fibroblasts	Regulation of intracellular iron levels, involved in the process of ferroptosis	[79]
FSP1	Synovial cell	Inhibition of ferroptosis by non-GPX4 pathway	[80]
NOX4	Synovial cell	Produce ROS, positively regulate ferroptosis	[23]
ZIP8	Synovial cell	Regulating zinc ion transport and affecting ferroptosis	[81]
SIRT1	Synovial cell	Regulate ferroptosis by deacetylation	[82]
NRF2	Synovial cell	Regulation of ferroptosis by antioxidant response	[83]
P53	Synovial cell	Iron death can be triggered under certain conditions.	[72]
PTGS2	Synovial cell	Participate in the inflammatory process, indirectly affect ferroptosis	[84]
TNF	Macrophage, synovial cell	Regulation of inflammatory response, indirectly involved in the process of ferroptosis	[85]

Abbreviations: ACSL4, long-chain acyl-CoA synthetase 4; FSP1, ferroptosis suppressor protein 1; TFR1, transferrin receptor 1; NCOA4, nuclear receptor coactivator 4; NOX4, NADPH oxidase 4; ZIP8, japonica Zinc transporter 8; SIRT3, sirtuin 3; NRF2, nuclear factor (erythroid-derived 2)-like 2; P53, proteinp53; PTGS2, prostaglandin-endoperoxide synthase-2.

evolutionary conservation of ferroptosis-inflammation crosstalk—a paradigm likely conserved in human RA.[94] These antioxidants improve the metabolic environment of iron by scavenging free radicals in the body and reducing inflammation.[93] In addition, antioxidants can also enhance immunity, delay aging, and have a positive effect on improving the overall health status of RA patients. We can effectively improve the symptoms and quality of life of RA patients through reasonable iron supplementation, the application of iron chelating agents and the adjuvant treatment of antioxidants.

Cuproptosis

Mechanism of Cuproptosis

Cuproptosis is a newly discovered form of cell death characterized by the accumulation of intracellular copper ions and the regulation of protein lipoic acylation modification.[95] The process begins with the introduction of copper ions into cells via specific copper ion carriers, such as elesclomol, NSC-319726, and disulfide. These carriers disrupt the biosynthesis of iron-sulfur clusters and facilitate the direct influx of copper ions into the cells.[96] Once intracellular copper ions reach a critical threshold, they bind to lipoic acylated proteins, especially to dehydroacyl transacetylase (DLAT).[97,98] DLAT is a component of the pyruvate dehydrogenase complex (PDH complex), which is integral to the tricarboxylic acid cycle (TCA cycle) of cells.[99] The binding of copper ions to DLAT induces the hetero-polymerization of DLAT, leading to the formation of insoluble aggregates. These aggregates disrupt the normal function of DLAT, thereby blocking the TCA cycle.[99,100]

Moreover, copper ions also bind to Ferredoxin1 (FDX1).[101] FDX1 is a crucial regulator involved not only in cuproptosis but also as an upstream regulator of protein acylation.[102] When copper ions bind to FDX1, they alter their redox state, which subsequently enhances the acylation and isomerization of DLAT (Table 3). This interaction exacerbates the blockade of the TCA cycle,[103] leading to a further decline in cellular energy production. The disruption of the TCA cycle results in a decreased energy supply within the cell, inducing protein toxicity stress and ultimately triggering

Table 3 Mechanism of Cuproptosis-Related Genes

Gene	Function	Regulation Trend	Cell Type
FDX1	Involved in the regulation of protein lipoic acylation modification, reducing Cu2 + to more toxic Cu +, resulting in the inhibition of Fe-S cluster protein synthesis and inducing cell death.	↑	A variety of cell types, especially in cells dependent on oxidative phosphorylation for capacitation.
DLAT	Involved in the pyruvate dehydrogenase complex and catalyzes the decarboxylation of pyruvate to acetyl-CoA in the tricarboxylic acid cycle. Heteropolymerization leads to cytotoxicity.	↑	It mainly plays a role in cells that rely on mitochondrial respiration.
LIPT1	Involvement in fatty acid transfer may be related to cell metabolism and signal transduction of Cuproptosis.	↑	It plays an important role in cells with active fat metabolism.
DLD	Encodes dehydrogenase enzyme subunits, participates in mitochondrial complexes, and affects cell metabolism and energy production.	↑	It exists in mitochondria and plays a key role in energy metabolism.
PDHA1	The α subunit of the pyruvate dehydrogenase complex is involved in pyruvate metabolism and tricarboxylic acid cycle, affecting the glycolysis process.	↑	It is expressed in cells with active glycolysis and mitochondrial respiration
PDHB	The β subunit of the pyruvate dehydrogenase complex interacts with PDHA1 to maintain the function of the pyruvate dehydrogenase complex.	↑	In coordination with PDHA1, it is expressed in a variety of cells.

(*Continued*)

Table 3 (Continued).

Gene	Function	Regulation Trend	Cell Type
LAIS	Involved in the synthesis of lipoic acid, may be related to the metabolic process of Cuproptosis.	↑	It plays a role in cells that require lipoic acid for metabolism
MTF1	As a negative regulatory gene, affect the Cuproptosis process by regulating the balance of metal ions.	↓	It is expressed in a variety of cells and plays an important role in metal ion homeostasis.
GLS	As a negative regulatory gene, involved in the process of Cuproptosis by affecting glutamine metabolism.	↓	Expressed in cells with active glutamine metabolism
CDKN2A	As a negative regulatory gene, may affect cell proliferation and death in the process of Cuproptosis by regulating cell cycle.	↓	It plays an important role in proliferating active cells, such as cancer cells.

Abbreviations: LIPT1, lipoyltransferase 1; DLD, dihydrolipoamide dehydrogenase; PDHA1, Pyruvate dehydrogenase alpha 1; PDHB, pyruvate dehydrogenase B;LIAS, Recombinant Lipoic Acid Synthetase; MTF1, Metal-regulatory transcription factor 1;GLS, Glutaminase; CDKN2A, Cyclin Dependent Kinase Inhibitor 2A.

cell death.[104] Moreover, the binding of copper ions to lipoic acylated proteins can impact other copper-related metabolic processes, such as mitochondrial respiration and lipoic acid synthesis, further intensifying the process of cell death[105] (Figure 5). To inhibit cuproptosis, several strategies can be employed. Copper chelating agents, oxidative phosphorylation inhibitors, and substances that affect the electron transport chain and mitochondrial pyruvate uptake can be utilized.[106]

Through statistical analysis of the screened samples, it was found that the keywords of cuproptosis research mainly revolve around tumor microenvironment, cancer, immunotherapy, and prognosis. From the keyword network clustering graph, it can be seen that cuproptosis research mainly focuses on gene features, prognostic models, and programmed cell death (Figure 6).

By generating clustering labels for index words, the keyword clustering map of English literature Q =0.769 (> 0.3), S = 0.858 (> 0.7), suggesting that clustering is effective and reasonable. The main label contents discussed in the module are extracted, as shown in (Table 4). The hot topics of cuproptosis research are: gene signature; the prognostic model; programmed cell death; overall survival; hepatocellular carcinoma; tumor microenvironment; immune infiltration; immune microenvironment; long non-coding RNAs.

The Role of Cuproptosis in RA Immune Inflammatory Response

RA is a complex autoimmune disease. Its pathological features include persistent inflammatory response, synovial cell proliferation, and destruction of articular cartilage and bone. In this process, the balance of copper ions may be seriously disturbed, resulting in excessive accumulation in joint tissue or immune cells infiltrated into joints. The pathological process involves multiple factors and mechanisms.[107] This accumulation is driven by dysregulation of copper transporters in the RA synovial microenvironment: Pro-inflammatory cytokines (eg, TNF-α, IL-6) downregulate ATP7A/B (copper efflux pumps) while upregulating CTR1 (SLC31A1, copper importer), trapping copper within cells.[108,109] Six transmembrane epithelial antigen of the prostate 4 (STEAP4) is an integral membrane protein that functions as a metalloreductase. It reduces extracellular Cu2 − to Cu − and enhances the influx of copper ions. A variety of experiments have shown that the metal transporter has strong affinity for iron and copper, and there are conserved metal binding sites.[110–112]

In recent years, with the in-depth study of cell death, the relationship between cuproptosis mechanism and RA has gradually attracted attention.[27] Cuproptosis, as a unique way of cell death, depends on the accumulation of copper ions in cells and the interaction with specific proteins.[97] In the joint inflammatory environment of RA, there may be some factors that lead to excessive accumulation of copper ions in joint tissue or immune cells.[113,114] These excess copper ions do not

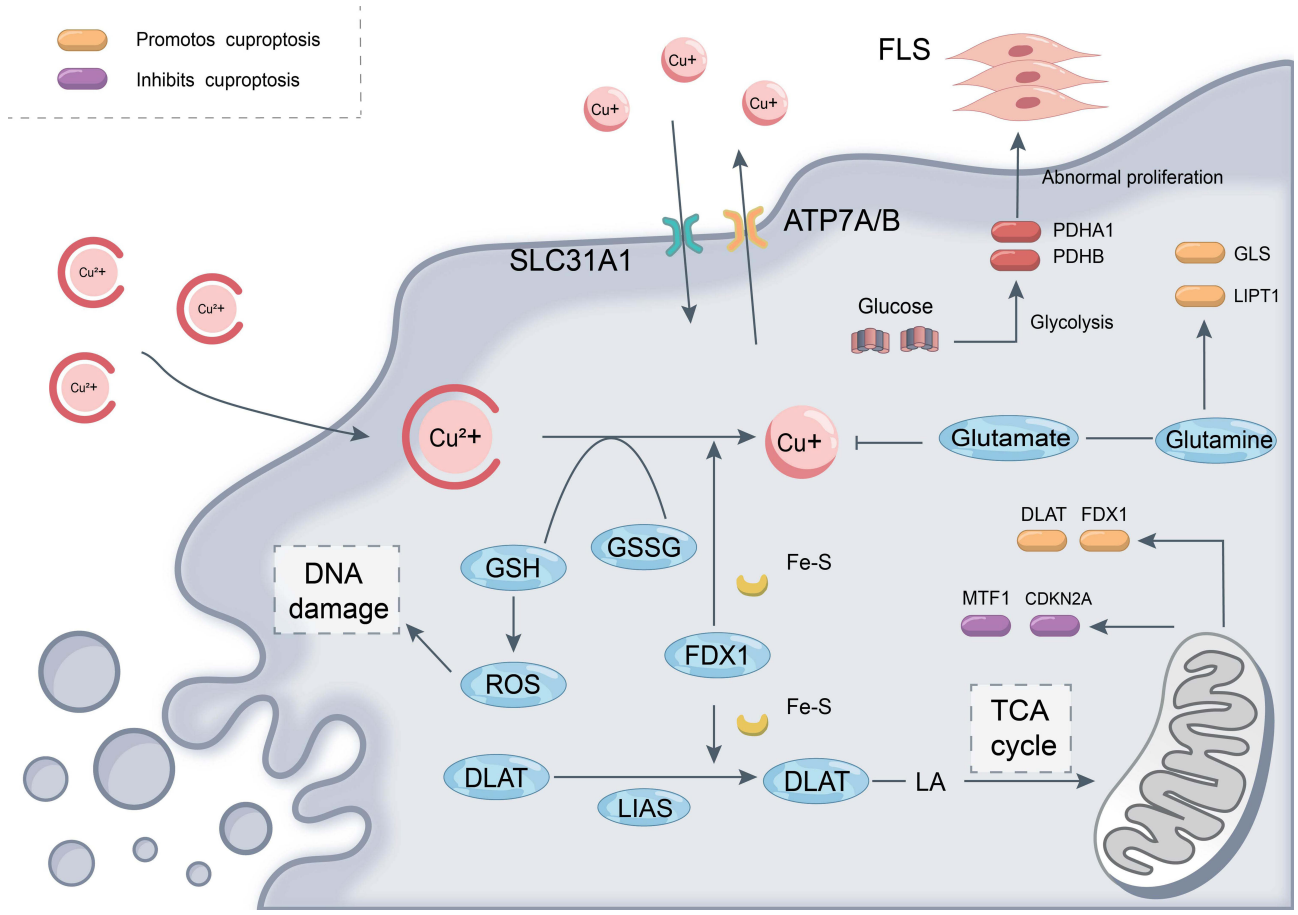

Figure 5 Signal transduction and mechanism of Cuproptosis. Cuproptosis enter cells through specific carriers, interfere with the synthesis of iron-sulfur clusters, and bind to lipoic acylated proteins, especially DLAT in PDH, causing their heteropolymerization into insoluble aggregates and hindering the tricarboxylic acid cycle. At the same time, copper ions bind to FDX1, change its redox state, aggravate DLAT acylation and isomerization, block the tricarboxylic acid cycle, reduce energy supply, induce protein toxic stress, and induce cell death. In addition, copper ion binding may also affect mitochondrial respiration, lipoic acid synthesis and other metabolic processes, exacerbating cell death. FLS, Fibroblast-like synoviocytes; ATP7a, Recombinant ATPase, Cu++ Transporting Alpha Polypeptide; ATP7b, Recombinant ATPase, Cu++ Transporting Beta Polypeptide.

Abbreviations: FDX1, Ferredoxin1; DLAT, dihydrolipoamide S-acetyltransferase; LIAS, lipoic acid synthetase; MTF1, metal regulatory transcription factor 1; CDKN 2A, cyclin-dependent kinase inhibitor 2A; PDHA1, Recombinant Pyruvate dehydrogenase alpha 1; PDHB, pyruvate dehydrogenase E1 subunit beta; LIPT1, lipoyltransferase 1; GLS, glutaminase.

exist in isolation. They actively seek to bind to intracellular proteins, particularly mitochondrial proteins such as FDX1 and DLAT. FDX1 reduces Cu^{2+} to Cu^{+}, displacing iron from Fe-S clusters (eg, in LIAS) and impairing mitochondrial respiration,[115,116] while copper binding to lipoylated DLAT triggers toxic aggregation, blocking the TCA cycle.[117] This process has a profound impact on the activation and function of immune cells. In particular, copper ions tend to form complexes with proteins that are closely related to mitochondrial respiratory chain and energy metabolism. As the ‘ energy factory ‘ of cells, the normal function of mitochondria is essential for maintaining cell survival. Syringaresinol-4-O-β-d-glucoside (SSG) is an effective inhibitor of macrophage-mediated inflammation and chondrocyte copper disease. Mechanistically, SSG inhibited the activation of NF-κB and NLRP3 pathways in MSU-induced macrophages. In addition, SSG regulates the expression of sulfur-linked mitochondrial enzymes (such as DLAT) in the cuproptosis pathway, thereby inhibiting the upstream regulator FDX1 in chondrocytes. SSG relieves inflammatory pain, which may be suitable for RA treatment.

The binding of copper ions to these key proteins may directly damage the structure and function of mitochondria by interfering with their normal conformation, enzyme activity or interaction network.[118] When copper ions bind to proteins in the cell, it may change the structure and function of these proteins.[27] These changes may lead to mitochondrial dysfunction, insufficient energy supply and accumulation of ROS (reactive oxygen species). Specifically, the binding of

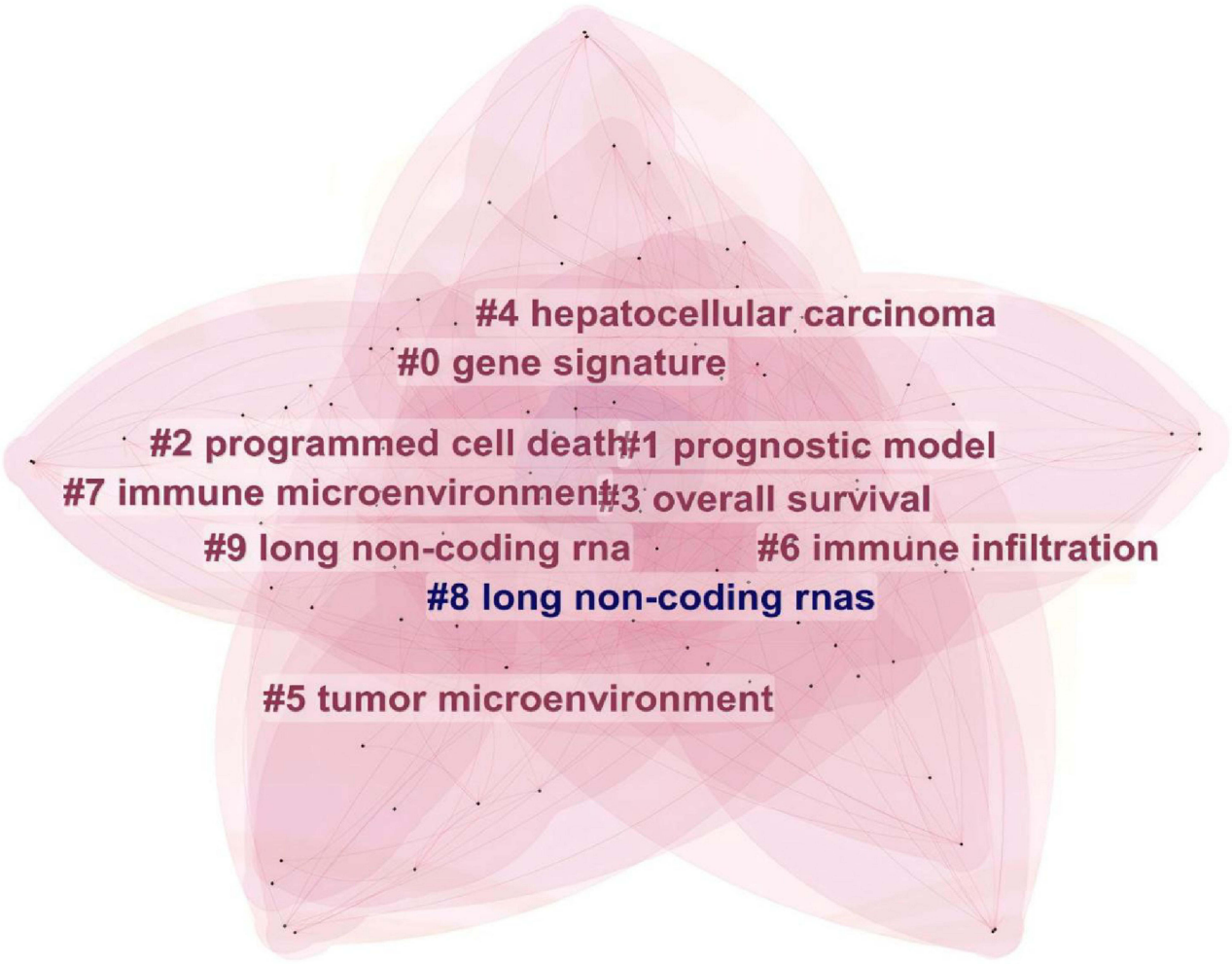

Figure 6 Cuproptosis keyword co-occurrence map.

copper ions may block the transmission of electrons in the mitochondrial respiratory chain, resulting in a decrease in ATP (adenosine triphosphate) synthesis, that is, the energy supply of cells is insufficient. This energy crisis further weakens the cell ‘s defense mechanisms, making it more difficult to cope with external stimuli and endogenous stress.

Mitochondrial dysfunction serves as a primary source of reactive oxygen species (ROS). Impaired mitochondrial handling of electron transport-generated free radicals (eg, superoxide anions) induces a sharp elevation in ROS, driving

Table 4 Cuproptosis Keyword Clustering Tags

Table	Year	Clustering Label Content
0#	2023	Gene signature; cuproptosis-related gene signature; transcription factor
1#	2022	Prognostic model; molecular subtypes; single-cell data analysis
2#	2021	Programmed cell death; hepatocellular carcinoma; immune microenvironment; cuproptosis-related gene signature
3#	2023	Overall survival; prognostic biomarker; comprehensive analysis; pancreatic adenocarcinoma; drug sensitivity
4#	2022	Hepatocellular carcinoma; tumor microenvironment; immune microenvironment; molecular subtypes
5#	2024	Tumor microenvironment; lung adenocarcinoma; tumor microenvironment; drug sensitivity;
6#	2024	Immune infiltration; immunological function; prognostic biomarker
7#	2023	Immune microenvironment; copper; disease protein; serum levels; mechanism
8#	2022	Long non-coding RNAs;bladder cancer; activatable imaging; lncrna immunotherapy; pancreatic adenocarcinoma

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oxidative stress. This stress exacerbates membrane lipid peroxidation and damages macromolecules (DNA, proteins, lipids), promoting apoptotic or necrotic cell death. In the RA synovial microenvironment, copper ion accumulation activates cuproptosis via binding to mitochondrial-associated proteins. This process amplifies local immune cell death while releasing DAMPs, which recruit/activate additional immune cells, establishing a pathogenic positive feedback loop that exacerbates joint inflammation and tissue destruction. Specifically, copper overload in RA synovial fluid disrupts mitochondrial respiration by binding to lipoylated TCA cycle proteins (eg, DLAT), triggering toxic aggregation and energy crisis.[27] Excess copper ions also interact with FDX1, a key regulator of cuproptosis, which reduces Cu^{2+} to cytotoxic Cu^{+}, exacerbating mitochondrial ROS production and synovial fibroblast dysfunction.[119] Furthermore, copper-induced aggregation of DLAT inhibits the pyruvate dehydrogenase complex, leading to ATP depletion and metabolic stress in RA fibroblast-like synoviocytes (FLS), thereby promoting inflammation and joint damage.[120] This process profoundly affects the activation and function of immune cells, aggravates the damage and inflammation of joint tissues, and provides a new perspective and therapeutic target for the pathological progress of RA.[121]

Furthermore, the mechanism of cuproptosis might also be linked to the autoimmune response in RA. RA is an autoimmune disease in which the immune system mistakenly attacks the joint tissues.[117] Copper ions can influence the activation and function of immune cells by binding to proteins within these cells. This interaction might enhance the immune cells' attack on joint tissues, exacerbating joint inflammation and injury. While the specific role of cuproptosis in RA remains not fully understood, exploring this mechanism might provide new insights and therapeutic strategies. A deeper dive into the relationship between the cuproptosis mechanism and RA might help better understand the pathogenesis of RA and develop effective treatment methods. Potential therapeutic approaches might include regulating the copper ions metabolism, inhibiting the process of cuproptosis, or interfering with copper-related signaling pathways. Such strategies aim to reduce joint inflammation and injury, thereby alleviating RA symptoms.[120]

Cuproptosis Gene Regulates RA Progression

Studies demonstrated that the expression of glutaminase (GLS1) was up-regulated in RA fibroblast-like synoviocytes, and silencing GLS1 with siRNA or inhibiting it with specific inhibitors could inhibit RA-FLS cell growth. Treatment with IL-17 or platelet-derived growth factors increased GLS1 levels in RA-FLS. The inhibition of GLS1 not only reduced the proliferation of RA-FLS but also diminished the inflammatory response in the arthritis mice model.[122] LIPT1 is another critical regulator in RA, primarily affecting mitochondrial respiration and lactic acid and pyruvate production. It is associated with synovial cell proliferation and angiogenesis in RA patients. In RA synovial tissues, the glycolytic activity is enhanced, creating an acidic microenvironment that promotes the transformation of normal synovial cells. This enhanced glycolysis is linked to the hypoxia of the RA synovial membrane. The key glycolytic enzymes, such as glucose phosphate isomerase, enolase, and aldolase, serve as autoantigens, contributing to RA immunity.[27,123] DLAT plays a role in RA by impacting mitochondrial function and the TCA cycle.[27] Additionally, *PDHB* has been identified as a susceptibility gene for RA, potentially, co-regulating Treg cells with Parkinson's disease protein 7 to maintain their functional integrity. The down-regulation of PDHB expression levels might lead to abnormal proliferation of RA-FLS.[27] FDX1, although not extensively studied in RA, is known to promote glycolysis and fatty acid oxidation, affecting amino acid metabolism and participating in inflammatory response.[124] LIAS is mainly related to mitochondrial function, oxidative stress, and inflammation. The overexpression of LIAS can increase the number of regulatory T cells (Treg), reduce T cell infiltration, inhibit NF-κB activity, reduce chronic inflammation, and protect mitochondrial function[27,125,126] (Figure 7).

Discussion

The formation of immune inflammation in RA involves a variety of mechanisms, including joint immune microenvironment and the participation of a variety of immune cells.[127] Changes in the microenvironment, such as the emergence of hypoxic and acidic environments, significantly promote the infiltration and activation of inflammatory cells, thereby exacerbating the formation and development of chronic inflammation.[128] Specifically, macrophages, as antigen-presenting cells, are crucial in RA. They not only engulf and process foreign antigens, but also present antigens to T cells through MHC molecules. While removing pathogens, macrophages may encounter oxidative stress, triggering

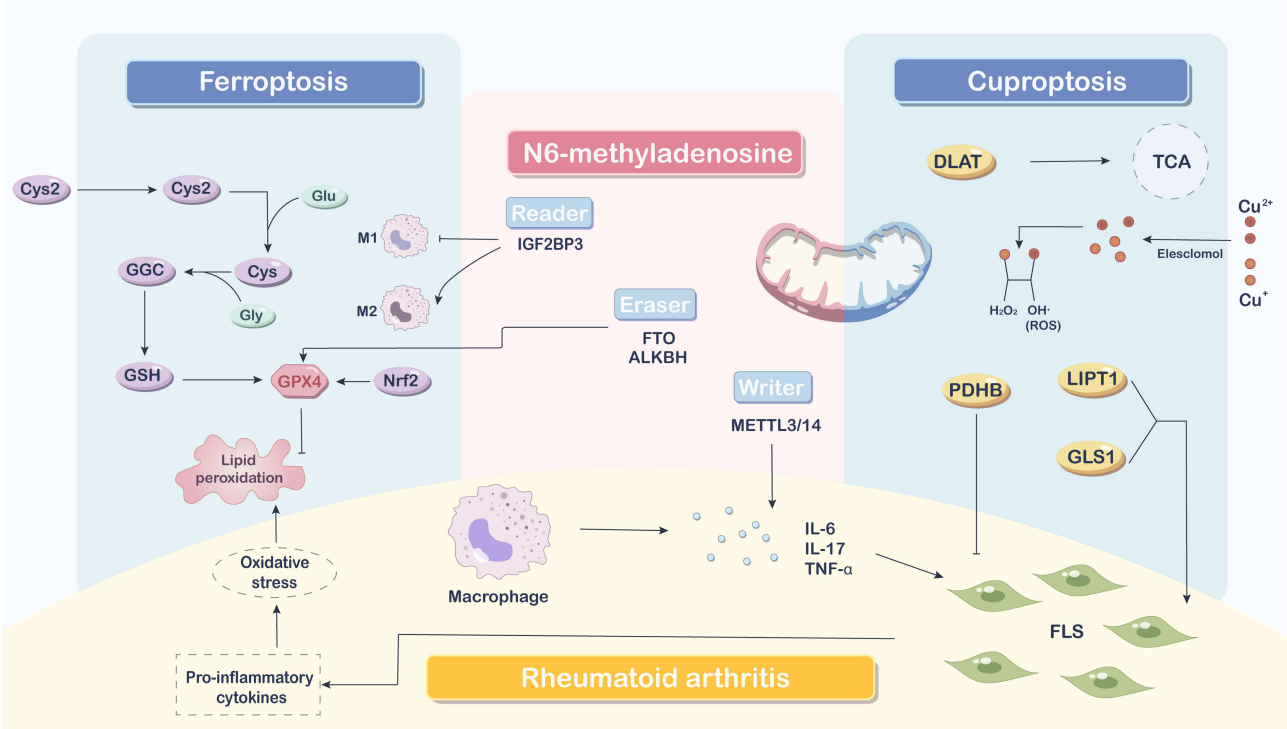

Figure 7 Ferroptosis and cuproptosis affect the immune inflammatory response of rheumatoid arthritis through m6A methylation. The m6A modification affects the production and release of inflammatory mediators by regulating the expression of inflammation-related genes, thereby indirectly regulating the role of ferroptosis and cuproptosis in inflammation. Pro-inflammatory factors secreted by immune cells and FLS in RA synovium promote oxidative stress, which further aggravates the inflammatory response and promotes cell death through this vicious cycle.

subsequent immune responses and ferroptosis.[129] At the same time, T cells are infiltrated in the joints of RA lesions, and are activated after recognizing antigens, releasing a variety of cytokines and mediators, further promoting the inflammatory response and joint damage.[130] Although the number of B cells is relatively small in the joint, activated plasma cells can generate autoantibodies such as rheumatoid factors, which bind to antigens to form immune complexes that deposit on the synovial membrane of the joint and induce an inflammatory response.[131] In addition, the number of mast cells in the synovium of RA is significantly increased, which significantly aggravates the inflammatory response and joint destruction process by secreting cytokines and mediators such as TNF- α and IL-1 β.[132]

Ferroptosis and cuproptosis have emerged as significant areas of study in cell biology, offering new insights into the complexity and diversity of cell death. While ferroptosis is driven by iron-dependent lipid peroxidation, cuproptosis hinges on copper-induced mitochondrial dysfunction. Intriguingly, both pathways converge on oxidative stress amplification in RA synovium. However, ferroptosis predominantly affects macrophages and fibroblasts, whereas cuproptosis selectively targets metabolically hyperactive T cells, suggesting cell-type-specific vulnerabilities. Notably, FDX1—a key regulator of cuproptosis—reduces Cu^{2+} to cytotoxic Cu^{+}, further exacerbating mitochondrial dysfunction. In RA-FLS, FDX1 upregulation correlates with enhanced glycolysis and oxidative stress, suggesting a metabolic shift toward cuproptosis susceptibility.[133] Recent evidence also highlights that copper overload in RA synovial fluid exacerbates mitochondrial ROS production, creating a feedforward loop between cuproptosis and ferroptosis.[134]

One key regulation mechanism that has been linked to both ferroptosis and cuproptosis is m6A methylation modification. This epigenetic modification plays a crucial role in gene expression regulation, affecting the sensitivity of immune cells to these forms of cell death by modulating the expression of immune-related genes.[98,135] Specifically, m6A modification may regulate the balance of iron or copper ions in immune cells and the activity of antioxidant defense system by enhancing or inhibiting the mRNA stability or translation efficiency of certain key genes (such as GPX4, SLC7A11, etc., which are related to ferroptosis, and specific genes that may be related to cuproptosis), thereby determining whether immune cells are prone to ferroptosis or cuproptosis. For instance, extensive research has

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illuminated that methyltransferases, notably METTL3, facilitate the expression of genes integral to ferroptosis via the modulation of m6A methylation, consequently expediting the progression of ferroptosis.[15] What's more, METTL3-mediated m6A methylation of GPX4 mRNA enhances its translation, thereby suppressing ferroptosis in RA-FLS.[136] Conversely, ALKBH5 demethylates SLC7A11 mRNA, destabilizing it and promoting ferroptosis—a process exacerbated by hypoxia in RA synovium.[137] Similarly, m6A readers like IGF2BP3 stabilize transcripts of cuproptosis-related genes (eg, PDHB), linking RNA metabolism to copper-induced cell death.

Furthermore, demethylases, including ALKBH5, occupy a pivotal position in regulating the landscape of m6A modifications. By erasing m6A marks from mRNAs, they exert a profound impact on gene expression patterns and cellular functionalities. In the context of ferroptosis, ALKBH5 and similar demethylases may safeguard cells against this form of cell death by modulating the m6A modification status of specific genes, thereby modulating iron homeostasis and enhancing antioxidant defenses.[44] Copper, an indispensable trace element for biological function, can turn detrimental when present in excessive amounts, eliciting cytotoxic effects. The m6A modification machinery may indirectly influence the manifestation of copper-induced cell death by fine-tuning the expression of genes implicated in copper transport, metabolism, and maintenance of its equilibrium. For example, METTL16, among other methyltransferases, may alter the stability or translational efficiency of mRNAs encoding copper metabolism proteins through its m6A-mediated activities.[138] Moreover, certain m6A reader proteins, such as YTHDFs, potentially contribute to the orchestration of cuproptosis-associated gene expression. These proteins discern and engage with m6A-tagged mRNAs, subsequently modulating their translational output or degradation rates. This, in turn, influences the synthesis of crucial proteins and ultimately governs the occurrence of copper-mediated cell death.[42,44] Thus, this intricate interplay between m6A modification, ferroptosis, copper homeostasis, and the involvement of various regulatory enzymes and proteins underscores the complexity of these cellular processes.

Immune cells are vital for maintaining homeostasis and resisting the invasion of external pathogens.[139] Ferroptosis and cuproptosis, as specialized forms of cell death, significantly influence the fate and function of these immune cells. By regulating the level of m6A modification, the proliferation, differentiation, and function execution of immune cells can be impacted, thereby modulating the overall response of the immune system.[38] In addition, m6A modification may also affect the gene expression of immune cell signal transduction pathways, such as NF-κB, MAPK and other key components of signaling pathways, to regulate the activation of immune cells, cytokine secretion and intercellular interaction, thus indirectly affecting the occurrence of ferroptosis and cuproptosis.[46] For instance, during immune cell activation, m6A modification can promote or inhibit ferroptosis and cuproptosis by regulating the expression of specific genes. This regulation affects the survival and activity of immune cells.[140]

The interplay between m6A RNA methylation, ferroptosis, and cuproptosis within the immune-inflammatory axis of RA remains an underexplored frontier. As a pivotal epigenetic mechanism governing post-transcriptional regulation, m6A methylation has been shown to dynamically regulate mRNA stability and translational efficiency of transcripts encoding key mediators of ferroptosis (eg, GPX4, ACSL4) and cuproptosis (eg, FDX1).[141–143] Mechanistically, m6A modifications on iron/copper homeostasis regulators – including metal transporters (eg, SLC40A1, SCL50A1) and metabolic enzymes (eg, LOX) – may critically disrupt intracellular redox balance by modulating metal ion flux and mitochondrial respiration, thereby driving these regulated cell death modalities.[144–146] Moreover, emerging evidence suggests that ferroptosis and cuproptosis are not merely passive cellular demise processes but active contributors to RA pathogenesis. The lytic release of DAMPs during these death programs – including mitochondrial DNA, HMGB1, and lipid peroxidation byproducts – engages pattern recognition receptors (eg, TLR4, NLRP3) on synovial macrophages and dendritic cells, potentiating immune cell activation and amplifying production of IL-1β, TNF-α, and other pro-inflammatory cytokines.[70] This self-reinforcing crosstalk between epitranscriptomic regulation and immunogenic cell death pathways sustains the chronic inflammatory milieu characteristic of RA.

The triad of m6A methylation, ferroptosis, and cuproptosis may establish a self-amplifying pathogenic loop in RA. Mechanistically, m6A epitranscriptomic modifications regulate iron/copper homeostasis by controlling the stability or translational efficiency of metal metabolism genes (eg, SLC7A11 for cystine/glutamate transport in ferroptosis; ATP7A for copper efflux in cuproptosis), thereby modulating susceptibility to these cell death modalities.[147] Conversely, ferroptosis-derived lipid peroxides and cuproptosis-liberated copper ions act as redox stressors that impair the activity

of m6A regulatory enzymes. This reciprocal regulation creates a feedforward cycle that globally reshapes the RA synovial transcriptome, amplifying pro-inflammatory cytokine production (eg, IL-6, IL-1β) and sustaining fibroblast-like synoviocyte hyperplasia.

Conclusion

Briefly, ferroptosis and cuproptosis have critical roles in immunity, inflammation, and RA by regulating m6A methylation modification. As a key mechanism of gene expression regulation, m6A modification significantly impacts the cell death process, thereby profoundly influencing immune system function, balance of inflammatory responses, and pathogenesis of RA. By thoroughly investigating the interaction mechanisms between m6A modification and ferroptosis and cuproptosis, new ideas and strategies can be developed for treating related diseases. Future studies should aim to identify the specific targets of m6A modification in the process of cell death, providing a foundation for precise disease treatment. Additionally, highlighting the relationship between m6A modification and other forms of cell death might offer a more comprehensive understanding of the regulatory network governing cell death.

Deficiency and Outlook

There are insufficient studies on the effects of ferroptosis and cuproptosis on RA through m6A methylation. The underlying mechanisms remain largely unknown. While the preliminary discussion of these concepts in RA has begun, the specific molecules, signaling pathways, and their interactions still require extensive research. Most current studies are speculative based on preliminary observations, lacking direct evidence. Moreover, clinical studies are scarce, necessitating more clinical data to elucidate the actual role of ferroptosis, cuproptosis, and m6A in RA patients.

Despite the shortcomings, the study of ferroptosis and cuproptosis affecting RA through m6A remains promising and full of potential. Future research should focus on identifying m6A-modified cuproptosis-related genes through integrated m6A-seq and RNA-seq analyses to delineate their epigenetic regulatory network in RA. Concurrently, the spatiotemporal heterogeneity of ferroptosis and cuproptosis across different RA stages should be systematically investigated using clinical synovial tissue samples and 3D organoid models to elucidate their synergistic roles in inflammatory microenvironment remodeling and bone erosion. Furthermore, therapeutic strategies targeting crosstalk nodes between m6A modification and iron/copper metabolic pathways should be developed, such as nanoparticle-based targeted delivery systems combining m6A demethylase inhibitors with copper chelators. Mechanistically, CRISPR-Cas9-mediated gene editing coupled with multi-omics approaches will unravel the molecular cascade by which the m6A-iron/copper axis regulates synovial fibroblast activation and chondrocyte pyroptosis. These efforts will establish a multidimensional theoretical framework and advance precision medicine for RA management.

Looking ahead, prioritizing biomarker-driven trials to stratify RA patients based on synovial iron/copper levels, developing dual-target inhibitors (eg, ACSL4/FDX1 inhibitors) to concurrently block ferroptosis and cuproptosis pathways, and engineering mitochondria-targeted nanocarriers (eg, liposome-encapsulated DFO) to enhance synovial specificity while minimizing systemic toxicity could accelerate clinical translation. These strategies, combined with mechanistic studies elucidating immune cell-specific susceptibility to metal-dependent cell death, may redefine precision medicine in RA.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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