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# Exploring the Mechanism of Curcumin's Impact on Cuproptosis Pathway in the Treatment of Rheumatoid Arthritis Based on Bioinformatics and Molecular Dynamics

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## Abstract

**Aim:** Rheumatoid Arthritis (RA) is a chronic inflammatory disease characterized by the abnormal proliferation and invasion of Rheumatoid Arthritis Fibroblast-Like Synoviocytes (RA-FLS). This study aimed to investigate the potential role of cuproptosis in the pathogenesis of RA and explore the impact of curcumin on cuproptosis-related genes as a novel therapeutic avenue for RA.

**Methods:** The GSE94648 dataset was obtained from the GEO database, and Gene Set Enrichment Analysis (GSEA) was performed to assess the expression of cuproptosis-related pathways. Cuproptosis-related genes were extracted in RA. Potential curcumin target genes were predicted through SwissTargetPrediction and Similarity Ensemble Approach websites, and the intersection with cuproptosis-related genes provided potential target genes for curcumin treatment. Molecular docking was carried out to analyze the interaction between curcumin and the potential target genes, followed by molecular dynamics simulations.

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Copyright © 2023 Xinpeng C. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. **Results:** In RA, the cuproptosis-related pathways were upregulated, and the expression of cuproptosis-related gene NFE2L2 was elevated in RA synovial tissues. Molecular docking studies indicated a strong binding affinity between curcumin and NFE2L2, which was maintained during molecular dynamics simulations.

**Conclusion:** Curcumin may influence NFE2L2 activity by regulating the cuproptosis pathway, providing a potential mechanism for the treatment of RA.

#### Keywords: Rheumatoid arthritis; Cuproptosis; Curcumin; Molecular Dynamics

## Introduction

Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory disease characterized by joint pain, swelling, and persistent morning stiffness [1]. RA has now become one of the most common causes of severe disability [2]. The interplay of environmental, genetic, and immunological factors has been shown to play a significant role in the development of RA [3]. Despite the widespread use of conventional and biologic anti-rheumatic drugs, approximately 5% of refractory RA patients show no response to current treatment methods [4]. Early diagnosis and timely treatment of RA can effectively slow disease progression and significantly reduce the occurrence of disability [5]. Therefore, the screening of diagnostic genes associated with RA, elucidation of the pathogenesis of RA, and the identification of new mechanisms at the molecular level offer potential avenues for the prevention and treatment of RA, possibly providing novel approaches to clinical therapy.

Cuproptosis is a recently discovered form of programmed cell death mediated by protein lipidation, closely linked to mitochondrial metabolism regulation [6]. Copper ions (Cu2+) are reduced to Cu through Ferredoxin 1 (FDX1), promoting the lipidation of mitochondrial proteins and the excessive production of key enzymes associated with the TCA cycle, thereby regulating crucial biological processes [7]. Copper is an essential cofactor and micronutrient for all living organisms, but excess levels can lead to cell death. Previous studies have indicated that serum copper levels can serve as an indicator of RA disease activity [8]. Another study suggests that the relationship between cuproptosis and RA may be multifaceted, with various immune cells showing neurogenic

proliferation due to the suppression of critical neurogenic regulatory genes in various RA processes [9]. This indicates the potential of cuproptosis as a new therapeutic target, although current research on the relationship between cuproptosis and RA is relatively limited.

Chinese herbal medicine contains abundant natural compounds and is characterized by lower toxicity, making it a current focus of research for treating RA. Curcumin is a natural antioxidant that protects cells from inflammatory damage and has therapeutic effects on diseases such as myocardial, renal, and hepatic injuries [10-12]. However, its specific mechanisms still require further investigation.

This study employs bioinformatics to identify key genes related to the onset of RA and cuproptosis and explores the mechanism by which curcumin influences cuproptosis for the treatment of RA through molecular docking and molecular dynamics.

## **Materials and Methods**

#### Data retrieval

Using "Rheumatoid arthritis" as the keyword, a search was performed in the Gene Expression Omnibus database (www.ncbi. nlm.nih.gov/geo), resulting in the acquisition of the GSE94648 dataset, which comprises 28 healthy control samples and 152 samples from individuals with Rheumatoid Arthritis (RA). The detection platform for this dataset is GPL11154.

#### **GSEA** analysis and differential analysis

Based on literature, genes related to cuproptosis (NFE2L2, NLRP3, ATP7B, ATP7A, SLC31A1, FDX1, LIAS, LIPT1, LIPT2, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, CDKN2A, DBT, GCSH, DLST) were analyzed using the GSEA software (version 4.03). A random combination of 1,000 iterations was performed, with a statistical significance set at a False Discovery Rate (FDR) <0.05. The expression profiles of the 19 identified cuproptosis-related genes were extracted and subjected to differential analysis using the R software limma package.

#### Prediction of curcumin target genes

The molecular structure of curcumin was input into the online databases SwissTargetPrediction and similarity ensemble approach to predict its target sites. After obtaining the corresponding target genes, SwissTargetPrediction retained target genes with a probability greater than 0.1, while Similarity ensemble approach retained target genes with a MaxTC (Tanimoto Coefficient) correlation greater than 0.5. The intersection of the two prediction results with the differentially expressed genes obtained in step 1.2 yielded the potential target genes of curcumin, which were then used to construct a Venn diagram.

#### Molecular docking

The 3D structures of the targets were downloaded from the RCSB PDB database and uploaded to the PlayMolecule website to predict the docking pockets of the target proteins. The molecular structure of curcumin was retrieved from the PubChem database and imported into ChemBio3D Ultra 14.0 for energy minimization. The spatial coordinates of the docking site with the highest score predicted by the PlayMolecule website were input into the AutoDock Tools 1.5.6 software, and docking was performed using AutoDock Vina 1.1.2, with the ligand with the lowest binding energy selected as the best ligand.

#### Molecular dynamics simulation

Molecular dynamics simulations were conducted using the

Gromacs 2019.5 version software. The active small molecule was converted into a Gro-format molecular structure file via the ATB website, and the protein was also converted into a Gro-format molecular structure file, generating the topology file of the complex. Gromos 54A7\_atb force field and SPC water molecule model were used, and the system charge was neutralized. The entire simulation process was set at a temperature of 300 K. Prior to the simulation, the system was subjected to mechanical optimization using the steepest descent method. Subsequently, the system was equilibrated in the NVT and NPT ensembles, and a 20 ns molecular dynamics simulation was performed. Based on the dynamics results, the binding free energy of the receptor and ligand was calculated using g\_mmpbsa. Changes in the position and hydrogen bonding of the small molecule before and after the simulation were compared, and the Root Mean Square Deviation (RMSD), Radius of gyration (Rg), and Solvent-Accessible Surface Area (SASA) of the protein-small molecule complex were evaluated. The reasonableness of the protein structure after simulation was assessed using a Laue plot.

## Results

#### Analysis of differential cuproptosis-related genes

The differential analysis revealed that among 19 cuproptosisrelated genes, 9 genes exhibited differential expression (SLC31A1, ATP7A, GLS, NFE2L2, FDX1, DLAT, DLD, LIPT1, NLRP3), all of which were upregulated (Figure 1).

#### **GSEA** results

GSEA analysis results showed that cuproptosis-related pathways were upregulated in RA, with an FDR of less than 0.05 (Figure 2).

### Curcumin targets and potential targets for treating RA

SwissTargetPrediction predicted 67 potential target proteins for curcumin, while the similarity ensemble approach website predicted 28 potential target proteins. The intersection of these predictions with the results in section 2.1 identified one potential target for curcumin in treating RA, which is NFE2L2 (Figure 3).

#### Molecular docking results

Molecular docking results demonstrated that the binding energy between curcumin and NFE2L2 was -8.2 kcal/mol<sup>-1</sup>. These results indicate that curcumin binds to NFE2L2 through hydrogen bonds and hydrophobic interactions, demonstrating a strong binding affinity (Figure 4A).

#### Molecular dynamics simulation results

Molecular dynamics simulation revealed that the RMSD of the







curcumin-NFE2L2 complex reached stability at around 10 ns (Figure 5A). The Rg results showed a gradual reduction in the radius of the complex over the simulation time. SASA results indicated a decreasing trend in the complex's solvent-accessible surface area (Figure 5C). The docking results showed that the volume of the protein pocket reduced from 590.388 Å<sup>3</sup> before the simulation to 434.822 Å<sup>3</sup> after 20 ns. The complex maintained more than 2 hydrogen bonds



Figure 4: Molecular docking results before and after molecular dynamics simulation (Left side: White molecule represents the pre-simulation position, red molecule represents the post-simulation position; Right side: A) Molecular docking results of curcumin with NFE2L2 before simulation, B) Molecular docking results of curcumin with NFE2L2 after simulation).



throughout the simulation. Comparison with the pre-simulation structure revealed no significant changes in the position of the active small molecule, and curcumin and NFE2L2 were closely bound within the active pocket through hydrogen bonds and hydrophobic interactions, indicating stable binding (Figure 4B). The Laue plot is a fundamental indicator for assessing the reasonableness of the modeled structure, and the results showed that 98.5% of amino acid residues fell within the reasonable range, confirming the reliability and rationality of the protein structure after simulation (Figure 6). Calculations demonstrated that the total binding free energy between curcumin and NFE2L2 was -90.178  $\pm$  11.097 kJ/mol. The interactions were primarily driven by van der Waals forces, electrostatic potential, and nonpolar solvation effects, with polar solvation effects inhibiting binding (Figure 5, 6).

## Discussion

Rheumatoid Arthritis Fibroblast-Like Synoviocytes (RA-FLS),



as the predominant component of RA synovial tissue, play a pivotal role in the initiation and progression of RA. A substantial body of research has suggested that RA-FLS exhibits characteristics similar to those of tumor cells, such as excessive proliferation, migration, and invasiveness, making them a crucial factor in joint destruction. Overregulating the proliferation and migration of RA-FLS may present an effective approach to treating RA.

Cell apoptosis and necrosis represent the major forms of cell death, and recently, a novel form of cell death called cuproptosis has been described. It is characterized by excessive copper accumulation within the cell, resulting in the accumulation of mitochondrial acylated proteins and unstable Fe-S cluster proteins, ultimately leading to cell death [13]. A study found that active RA patients have higher serum copper levels compared to healthy individuals [14]. The copper levels in RA patients were positively correlated with DAS28 and ESR [15]. Another study indicated that elevated serum copper levels in RA patients could serve as early biomarkers for assessing disease activity [16]. Our research identified that cuproptosis-related pathways are upregulated in RA, implying that cuproptosis may play an important role in the pathogenesis of RA.

In this study, we observed upregulated expression of the cuproptosis-related gene NFE2L2. NFE2L2, also known as Nrf2, is a key transcription factor extensively involved in cellular antioxidant responses [17]. Studies have shown that NFE2L2 is upregulated in the synovium of RA [18]. NFE2L2 is the principal regulator of the cellular antioxidant response. Under normal conditions, NFE2L2 is bound to its inhibitor, KEAP1, and is localized in the cytoplasm. When cells undergo oxidative stress, NFE2L2 dissociates from KEAP1, translocates to the nucleus, and binds to Antioxidant Response Elements (ARE), thereby activating the gene expression of various antioxidant enzymes and cellular protective proteins [19]. RA is a

chronic inflammatory disease closely associated with oxidative stress. Research has revealed significant oxidative stress in RA patients, particularly in those with high disease activity, which may result in damage to cells and tissues [20]. In RA, after NFE2L2 activation, upregulation of antioxidant gene transcription occurs to suppress inflammation [21]. This suggests that NFE2L2 is one of the potential therapeutic targets in RA.

Curcumin, the main active component of turmeric, is a safe plant compound with a variety of biological activities, including antioxidant, anti-inflammatory, and anti-tumor activities. Li et al. reported that curcumin can improve mercury chloride-induced liver damage through the NFE2L2/ARE pathway [22]. Zhao et al. found that curcumin inhibits autophagic cell death through the PI2K/ AKT/NFE2L2 pathway, thereby ameliorating splenic damage [23]. Thus, it is evident that curcumin is a natural activator of NFE2L2. Excessive proliferation of RA Fibroblast-Like Synoviocytes (FLS) is a major cause of synovial tissue proliferation, and inhibiting synovial proliferation is a key aspect of its treatment. Studies have shown that curcumin can effectively suppress the inflammatory response in RA by inhibiting the production of pro-inflammatory mediators and regulating humoral and cellular immune responses [24]. Another study also found that curcumin has a therapeutic effect on rheumatoid arthritis by regulating the NFE2L2-Keap1 pathway [25]. This study, from a molecular docking and molecular dynamics perspective, revealed that curcumin can effectively bind to NFE2L2. The results indicate that after binding, certain regions of the protein become more compact, and the entire system experiences an increase in hydrophobicity and structural stability. Multiple results suggest that curcumin may activate NFE2L2, affecting the regulation of cuproptosis and, thereby, treating RA.

In summary, our study, based on bioinformatic analysis and molecular dynamics exploration, finds that curcumin can improve rheumatoid arthritis by influencing cuproptosis through the modulation of NFE2L2 activity in synovial tissue. However, this study still has certain limitations, and the detailed regulatory mechanisms of cuproptosis in RA require further investigation. Comprehensive clinical or experimental research is needed in the future to validate the interaction between the candidate gene NFE2L2 and curcumin.

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