

Original Article

ALKBH5 Regulates Apoptosis of Rheumatoid Arthritis Fibroblast-like Synoviocytes by Modulating miR-181b-5p Maturation via m6A Demethylation

Dexin Wang¹, Yu Fang¹, Rui Liu¹, Wensuo Long¹, Huaiming Deng¹, Liwei Yu¹, Dan Wang²¹Department of orthopedics, Haishu District People's Hospital, Ningbo, China;²Department of Pharmacology, Medical College, Dalian University, Dalian, China.**Abstract**

Objective: To investigate the role of ALKBH5, a member of the AlkB family of Fe(II)/ α -KG-dependent dioxygenases that functions as an RNA demethylase removing N6-methyladenosine (m6A), in regulating apoptosis of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS), with a focus on miR-181b-5p maturation. **Methods:** RA-FLS were isolated and analyzed for ALKBH5, pre-miR-181b-1, and miR-181b-5p expression using qRT-PCR. ALKBH5 protein levels were assessed by Western blotting. Cell proliferation and apoptosis were evaluated using MTT assay and flow cytometry, respectively. m6A modification on pre-miR-181b-1 was measured via MeRIP, and its binding to DGCR8 was assessed using co-immunoprecipitation. **Results:** ALKBH5 expression was significantly downregulated in RA-FLS. ALKBH5 overexpression inhibited proliferation and promoted apoptosis, while its knockdown had the opposite effect. ALKBH5 decreased m6A modification of pre-miR-181b-1, thereby increasing levels of pre-miR-181b-1 and mature miR-181b-5p. Inhibition of miR-181b-5p attenuated the effects of ALKBH5. **Conclusion:** Overexpression of ALKBH5 promotes apoptosis and inhibits proliferation in RA-FLS by demethylating m6A on pre-miR-181b-1, thereby enhancing miR-181b-5p maturation. These findings suggest a novel therapeutic target for rheumatoid arthritis.

Keywords: ALKBH5, Fibroblast-like Synoviocytes, miR-181b-5p, m6A Modification, Rheumatoid Arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by synovial inflammation, joint destruction, and a range of systemic complications. The pathogenesis of RA is multifactorial, involving genetic, epigenetic, and environmental components². Clinically, patients often present with morning stiffness, joint swelling, and pain³. If left untreated, RA can result in irreversible joint deformities, functional disability, and serious systemic manifestations, including cardiovascular complications⁴.

Despite significant advances in therapeutic strategies, a substantial proportion of patients continue to experience persistent disease activity.

Fibroblast-like synoviocytes (FLS) are central to RA pathogenesis. Their aberrant behavior plays a key role in sustaining chronic inflammation and promoting joint destruction^{5,6}. In RA, FLS acquire tumor-like features, including uncontrolled proliferation, resistance to apoptosis, and elevated secretion of pro-inflammatory cytokines⁷. These pathological traits contribute to synovial hyperplasia and the release of matrix-degrading enzymes, accelerating cartilage and bone erosion⁸. Although the involvement of FLS in RA has been well established, targeted therapies specifically aimed at correcting their dysfunctional behavior remain limited⁹.

ALKBH5, a member of the AlkB family of Fe(II)/ α -ketoglutarate-dependent dioxygenases, functions as an RNA demethylase that removes N6-methyladenosine (m6A) modifications. It plays a pivotal role in the post-transcriptional regulation of gene expression by influencing

The authors have no conflict of interest.

Corresponding author: Dan Wang, No. 10 Xuefu Street, Dalian, Economic and Technological Development Zone, Liaoning Province, China
E-mail: wangdan1@dlu.edu.cn

Edited by: G. Lyritis
Accepted 14 April 2025



RNA stability and function, particularly in non-coding RNAs^{10,11}. Recent studies have shown that ALKBH5 modulates the migration, invasion, and proliferation of RA-FLS through m6A-dependent mechanisms¹². However, the precise molecular mechanisms and downstream pathways by which ALKBH5 contributes to RA pathogenesis remain to be fully elucidated.

MicroRNAs (miRNAs) are small non-coding RNAs of approximately 18–22 nucleotides in length that post-transcriptionally regulate gene expression¹³. They have been shown to be critical in the pathogenesis of RA, influencing bone remodeling and modulating FLS function^{14,15}. In this study, we investigate whether ALKBH5 regulates the m6A modification of pre-miR-181b-1, thereby affecting its maturation and the functional role of miR-181b-5p in RA-FLS, ultimately contributing to RA progression.

Isolation and Culture of Fibroblast-like Synoviocytes

Rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) were isolated from synovial tissue samples obtained from 12 patients diagnosed with RA (mean age: 53.33 ± 5.57 years), based on the classification criteria of the American College of Rheumatology^{5,6}. The demographic and clinical characteristics of the RA patients are provided in Supplementary Table 1. Normal fibroblast-like synoviocytes (NL-FLS) were isolated from synovial tissue samples obtained from 11 patients undergoing joint surgery for non-inflammatory orthopedic conditions, with no prior history of arthritis. All samples were immediately stored at -80°C until further processing.

Synovial tissues were initially placed in serum-free medium, and all procedures were conducted under sterile conditions. The tissues were transferred into serum-free medium containing penicillin-streptomycin to eliminate fat and fibrous material. Subsequently, tissues were washed in sterile phosphate-buffered saline (PBS) to remove blood and debris. The samples were minced into approximately 2 mm pieces using autoclaved ophthalmic scissors and transferred to sterile culture dishes. Enzymatic digestion was performed using type I collagenase (0.5 mg/mL) diluted 1:300, incubated at 37°C for 3 hours. Following digestion, the cell suspension was filtered and centrifuged at 300 × g for 5 minutes to eliminate residual fat and debris, and the supernatant was discarded¹⁶.

The resulting cell pellet was resuspended and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 15% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, and maintained at 37°C in a humidified incubator with 5% CO₂. Cells from passages 3 to 8 were used for all subsequent experiments. Each experiment was repeated in triplicate. All samples were stored at -80°C for further use.

Cell Transfection

FLS were seeded at a density of 1 × 10⁶ cells/mL into 24-well plates. When cell confluence reached approximately 80%, transfection was performed using Lipofectamine 3000 (Thermo Fisher Scientific, USA), following the manufacturer's instructions. ALKBH5-specific siRNA (si-ALKBH5) and its negative control (si-NC), as well as a miR-181b-5p inhibitor and its corresponding negative control inhibitor, were synthesized by Shanghai GenePharma Co., Ltd (China) and used to silence target gene expression. For overexpression studies, an ALKBH5 expression plasmid and its corresponding empty vector were synthesized by Sangon Biotech (Shanghai) Co., Ltd (China).

After 6 hours of transfection, the medium was replaced with fresh DMEM containing FBS. Cells were harvested 48 hours post-transfection, and supernatants were collected for subsequent analyses.

Flow Cytometry Detection

Apoptosis in FLS was assessed using flow cytometry. Briefly, cells were digested with 0.25% trypsin and centrifuged at 1,000 × g for 10 minutes. The supernatant was discarded, and the cell pellet was resuspended in complete culture medium. Cells were seeded at a density of 1 × 10⁶ per well in 96-well plates.

Apoptosis was evaluated using the Annexin V-FITC/Propidium Iodide (PI) Apoptosis Detection Kit (BD Biosciences, USA). A 100 µL aliquot of the cell suspension was incubated with 5 µL of Annexin V-FITC and 5 µL of PI in the dark at room temperature for 20 minutes. After staining, apoptotic cells were analyzed by flow cytometry, and data were processed using FlowJo software (Version 10).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted using the mRNA extraction kit (K157002, Thermo Fisher Scientific, USA). RNA concentration and purity were assessed by measuring the A260/A280 ratio. Complementary DNA (cDNA) was synthesized from RNA via reverse transcription, following the protocol provided with the reverse transcription kit (R312-O1, Nanjing Vazyme Biotech Co., Ltd, China).

qRT-PCR was performed using the 7500 Fast Real-Time PCR System (BIO-RAD, USA). GAPDH served as the internal control. The PCR conditions were as follows: initial denaturation at 95°C for 3 minutes; 39 cycles of denaturation at 95°C for 10 seconds, annealing at 58°C for 30 seconds; and extension at 65°C for 5 seconds. Amplification and melting curves were confirmed for all reactions. Relative gene expression was calculated using the 2^{-ΔΔCT} method.

Primer sequences were as follows:

- **ALKBH5:**
 - Forward: 5'-CATCTCATCGATCCCGGTGG-3'

- Reverse: 5'-CACTGGCGAGGTCATAGGTC-3'
- *miR-181b-5p*:
 - Forward: 5'-CGCGACCCACCGACAGC-3'
 - Reverse: 5'-AGTGCAGGGTCCGAGGTATT-3'
- *pre-miR-181b-1*:
 - Forward: 5'-CTCGCTTCGGCAGCACA-3'
 - Reverse: 5'-AACGCTTACGAATTTGCGT-3'
- *GAPDH*:
 - Forward: 5'-TGGCTGGCTCAGAAAAGGG-3'
 - Reverse: 5'-GGGAGATTCAGTGTGGTGGGG-3'

Western Blot Analysis

Total protein was extracted from cells using a protein extraction kit (O24C1007, Shanghai Epizyme Biomedical Technology Co., Ltd). Protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Shanghai Beyotime Biotechnology Co., Ltd). Equal amounts of protein were subjected to electrophoresis using 12% precast polyacrylamide gels and transferred onto polyvinylidene fluoride (PVDF) membranes.

Membranes were blocked with 5% skimmed milk for 1.5 hours at room temperature. Subsequently, membranes were incubated overnight at 4°C with the following primary antibodies: ALKBH5 (ab195377), DGCR8 (ab191875), and m6A (ab208577). After three washes with Tris-buffered saline containing Tween-20 (TBST), membranes were incubated for 1.5 hours at room temperature on a shaker with horseradish peroxidase (HRP)-conjugated secondary antibodies—either goat anti-rabbit IgG (abs20002ss) or goat anti-mouse IgG (abs20039).

Following three additional TBST washes, the membranes were incubated with enhanced chemiluminescence (ECL) solution (sb-wb012, Shanghai Share-bio Biotechnology Co., Ltd; mixture of solution A and B) and visualized using a multifunctional imaging system (Vilber, Fusion FX5 Spectra, France). GAPDH was used as the internal loading control. The relative protein expression levels were quantified using ImageJ software (National Institutes of Health, USA) by measuring the gray value of the bands.

MTT Assay

Cell proliferation was evaluated using the MTT assay kit (M1020, Beijing Solarbio Science & Technology Co., Ltd), based on the reduction of MTT by mitochondrial enzymes in viable cells to form purple formazan crystals¹⁷. Cells were seeded into 96-well plates at a density of 2×10^3 cells per well in a final volume of 200 μ L per well. At the indicated time points, 10 μ L of MTT solution was added to each well, and the plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 3 hours. After incubation, the culture medium was carefully removed, and 150 μ L of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Plates were gently shaken for 10 minutes, and absorbance was measured at 570 nm using a microplate reader (Tecan, Switzerland). The data were recorded and analyzed accordingly.

Methylated RNA Immunoprecipitation (MeRIP) Assay

The MeRIP assay was conducted using the Methylated RNA MeRIP Kit (CR203146, Millipore, USA), following the manufacturer's instructions. Total RNA was extracted from RA-FLS and enzymatically fragmented to approximately 200 nucleotides in length. For immunoprecipitation, a DGCR8-specific antibody conjugated to protein A/G magnetic beads was first used to enrich RNA-protein complexes. Subsequently, an anti-m6A antibody was applied to selectively isolate m6A-modified RNA fragments.

A control immunoprecipitation using IgG was included to account for nonspecific antibody binding. The enriched RNA was then subjected to qRT-PCR to quantify specific RNA targets. Additionally, protein levels of DGCR8 and m6A-associated components in the immunoprecipitated complexes were analyzed via Western blotting to verify enrichment and modification status.

Statistical Analysis

All experimental data were analyzed using SPSS 23.0 statistical software. GraphPad Prism 9.0 was used for data visualization. Comparisons between two groups were performed using a two-tailed, unpaired Student's *t*-test. For comparisons among three or more groups, one-way analysis of variance (ANOVA) was employed. Data are expressed as mean \pm standard deviation (SD). Each experiment was independently repeated at least three times, with a minimum of three replicates per group. A *p*-value of < 0.05 was considered statistically significant.

Results

ALKBH5 Is Significantly Downregulated in RA-FLS

Given the established role of ALKBH5 as an m6A demethylase in various diseases¹⁸, we first assessed its expression in normal fibroblast-like synoviocytes (NL-FLS) and rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS). Both qRT-PCR and Western blot analyses demonstrated that ALKBH5 mRNA and protein levels were significantly reduced in RA-FLS compared to NL-FLS ($P < 0.05$) (Figure 1A, B).

Overexpression of ALKBH5 Inhibits Proliferation and Promotes Apoptosis in RA-FLS

To explore the functional role of ALKBH5 in RA-FLS, we overexpressed or silenced ALKBH5 and evaluated its effects on cell proliferation and apoptosis. As shown by qRT-PCR and Western blot, ALKBH5 expression was markedly increased in the overexpression group compared to the vector control, while it was significantly reduced in the si-ALKBH5 group compared to the si-NC group ($P < 0.05$) (Figure 2A, B).

MTT assays revealed that RA-FLS in the ALKBH5-overexpressing group exhibited significantly reduced

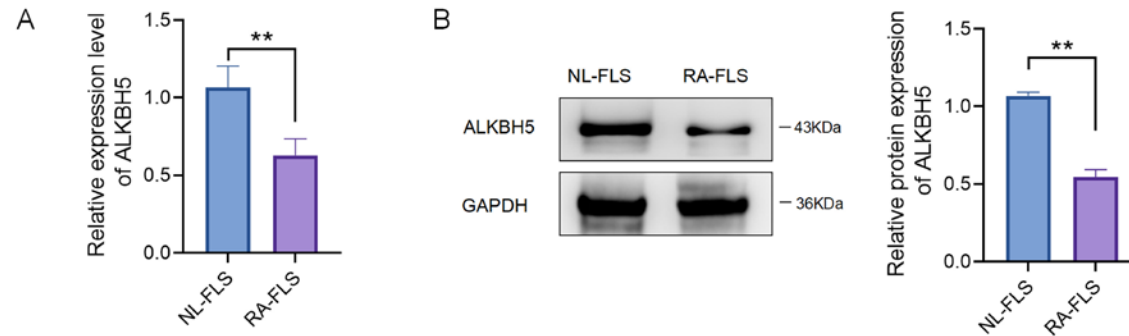


Figure 1. ALKBH5 is significantly down-regulated in RA-FLS (A) Relative gene expression of ALKBH5 in RA-FLS and NL-FLS measured by qRT-PCR; (B) Protein expression levels of ALKBH5 detected by Western blot. Data are presented as mean \pm SD (n=3). ** $P < 0.05$ vs NL-FLS. RA-FLS: Rheumatoid arthritis fibroblast-like synoviocytes; NL-FLS: Normal fibroblast-like synoviocytes; qRT-PCR: Quantitative real-time polymerase chain reaction; ALKBH5: AlkB homolog 5, an m6A RNA demethylase.

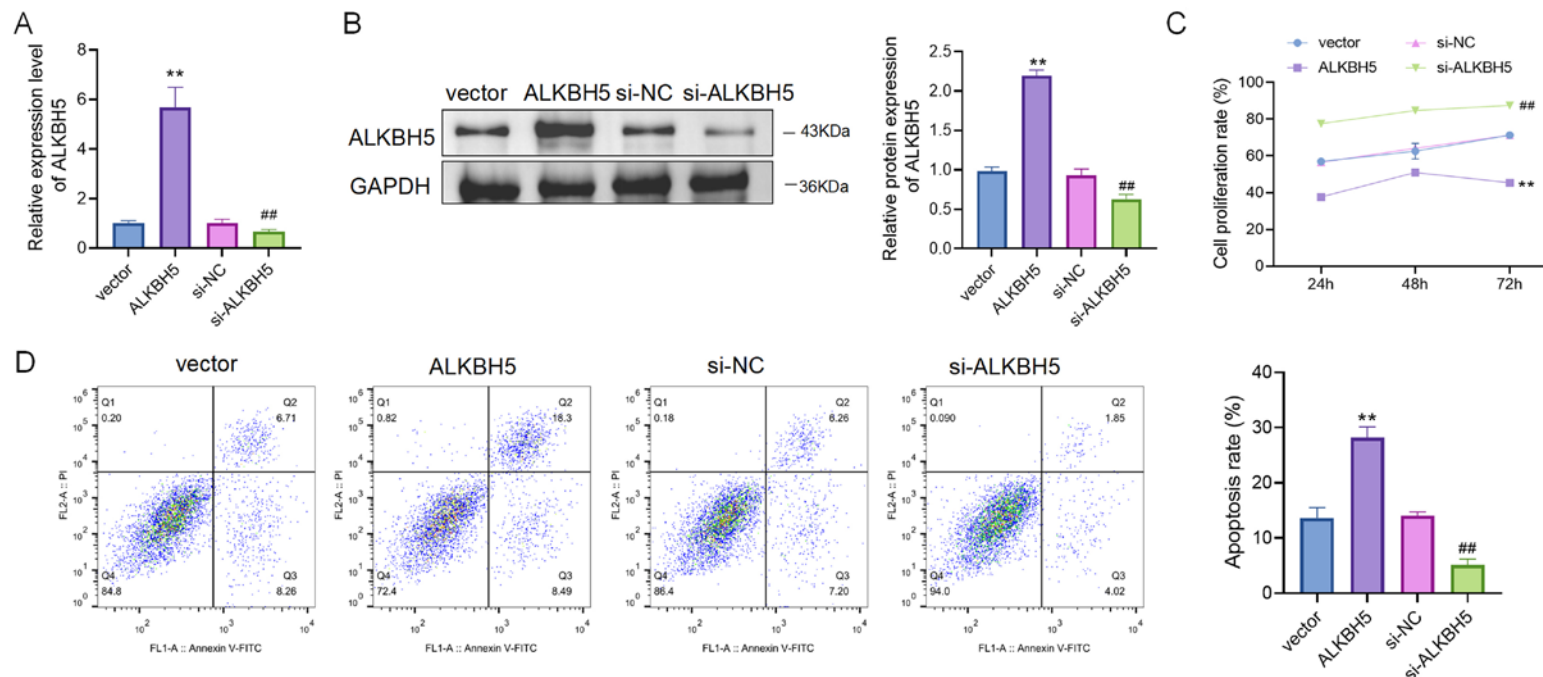


Figure 2. Overexpression of ALKBH5 in RA-FLS inhibits proliferation and promotes apoptosis (A) qRT-PCR analysis of ALKBH5 mRNA expression levels in RA-FLS; (B) Western blot analysis of ALKBH5 protein expression in RA-FLS; (C) MTT to determine the proliferation of RA-FLS at 24, 48, and 72h in each group; (D) Flow cytometry to examine the apoptosis level of RA-FLS. Data are presented as mean \pm SD (n=3). ** $P < 0.05$ vs vector group; ## $P < 0.05$ vs si-NC group. ALKBH5: AlkB homolog 5, an m6A RNA demethylase; RA-FLS: Rheumatoid arthritis fibroblast-like synoviocytes; qRT-PCR: Quantitative real-time polymerase chain reaction; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

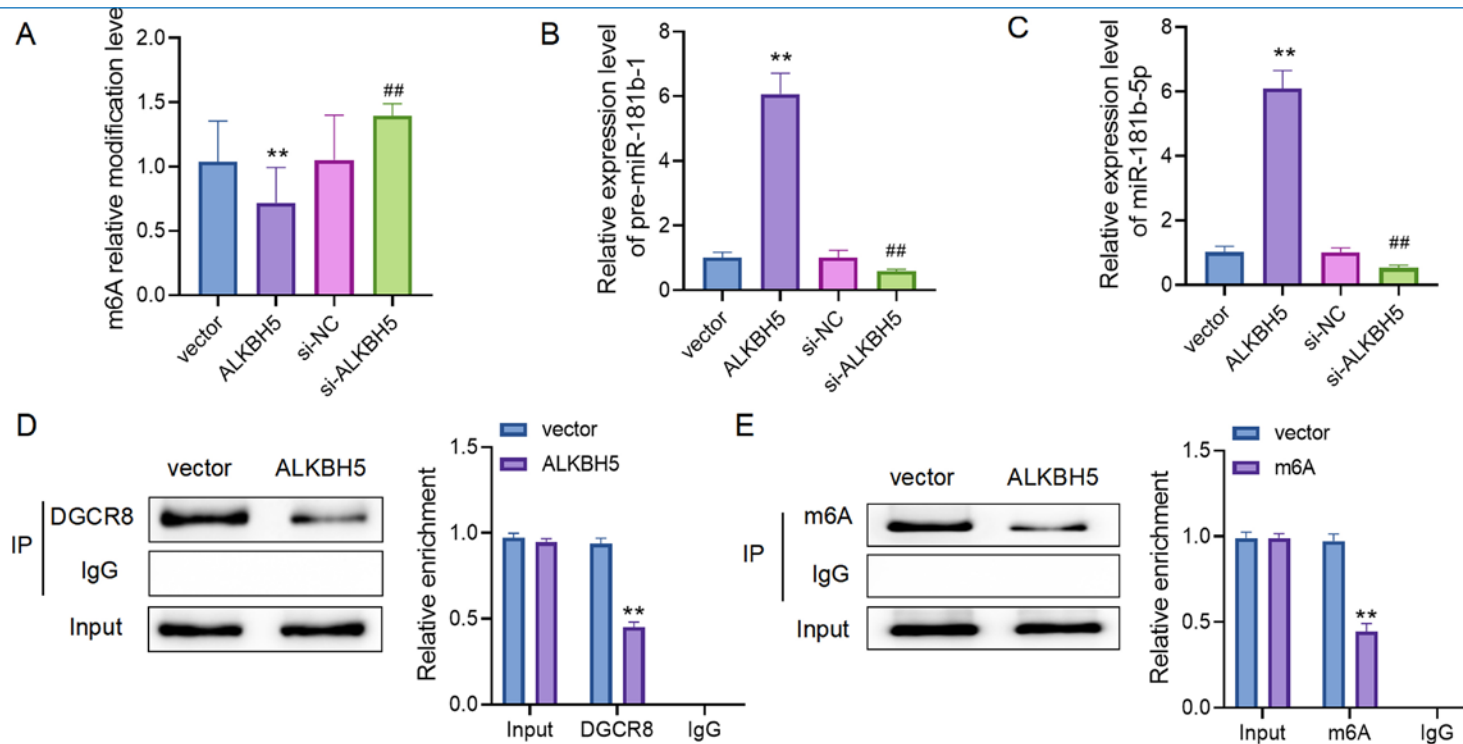


Figure 3. ALKBH5 up-regulates the expression of pre-miR-181b-1 and miR-181b-5p by reducing m6A modification (A) MeRIP-qPCR analysis to quantify m6A modification levels in RA-FLS; (B) qRT-PCR to measure the expression level of pre-miR-181b-1 and (C) miR-181b-5p in RA-FLS; (D) Immunoprecipitation to examine the binding of pre-miR-181b-1 to DGCR8 in RA-FLS; (E) Immunoprecipitation to analyze the level of m6A-modified pre-miR-181b-1 in RA-FLS. Data are presented as mean \pm SD (n=3). ** $P < 0.05$ vs vector group; *** $P < 0.05$ vs si-NC group. ALKBH5: AlkB homolog 5, an m6A RNA demethylase; m6A: N6-methyladenosine; MeRIP-qPCR: Methylated RNA immunoprecipitation-quantitative polymerase chain reaction; RA-FLS: Rheumatoid arthritis fibroblast-like synoviocytes; qRT-PCR: Quantitative real-time polymerase chain reaction; DGCR8: DiGeorge syndrome critical region gene 8.

proliferation compared to the vector group, whereas silencing ALKBH5 markedly enhanced cell proliferation compared to the si-NC group ($P < 0.05$) (Figure 2C).

Flow cytometry analysis further showed that apoptosis levels were significantly increased in the ALKBH5-overexpression group relative to the vector control. Conversely, apoptosis was significantly reduced in RA-FLS transfected with si-ALKBH5 compared to the si-NC group ($P < 0.05$) (Figure 2D).

ALKBH5 Enhances the Expression of pre-miR-181b-1 and miR-181b-5p by Reducing m6A Modification

To determine whether ALKBH5 regulates RA-FLS function through m6A

demethylation, we next evaluated m6A modification levels in transfected cells. MeRIP-qPCR analysis indicated that m6A levels were significantly decreased in the ALKBH5-overexpression group and significantly increased in the si-ALKBH5 group, compared with their respective controls ($P < 0.05$) (Figure 3A).

qRT-PCR results showed that expression levels of pre-miR-181b-1 and mature miR-181b-5p were significantly upregulated following ALKBH5 overexpression and downregulated upon ALKBH5 silencing ($P < 0.05$) (Figure 3B, C).

Previous studies have shown that m6A modification mediated by methyltransferase-like 3 (METTL3), an RNA methyltransferase, promotes the processing of pri-miRNAs such as pri-miR-19a via DGCR8 interaction¹⁹. Consistent

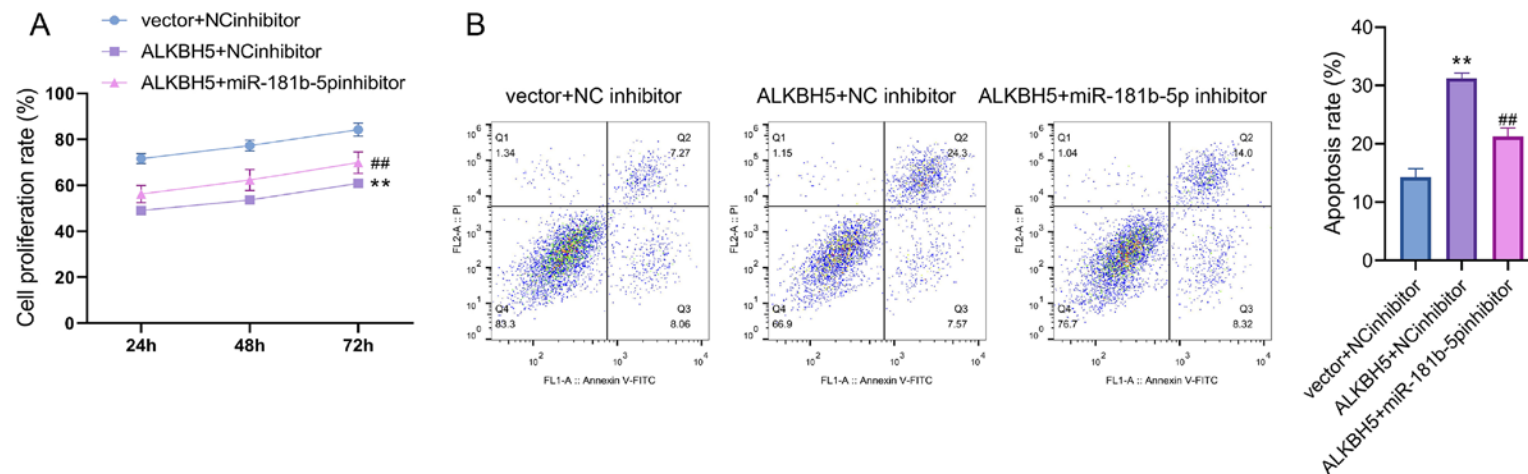


Figure 4. Down-regulation of miR-181b-5p expression inhibits the effects of ALKBH5 (A) MTT assay to evaluate the of RA-FLS at 24, 48, and 72h; (B) Flow cytometry assay for the apoptosis level of RA-FLS in each group. Data are presented as mean \pm SD (n=3). ** $P < 0.05$ vs vector + NC inhibitor group; ## $P < 0.05$ vs ALKBH5 + NC inhibitor group. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ALKBH5: AlkB homolog 5, an m6A RNA demethylase; RA-FLS: Rheumatoid arthritis fibroblast-like synoviocytes.

with this, co-immunoprecipitation analysis in our study revealed that ALKBH5 overexpression significantly reduced the binding of pre-miR-181b-1 to DGCR8 ($P < 0.05$) (Figure 3D). In parallel, the m6A modification level of pre-miR-181b-1 was also significantly reduced ($P < 0.05$) (Figure 3E).

Downregulation of miR-181b-5p Attenuates the Effects of ALKBH5 on RA-FLS

To determine whether the effects of ALKBH5 on RA-FLS are mediated through miR-181b-5p, we inhibited miR-181b-5p expression in the presence or absence of ALKBH5 overexpression. As shown in the results, cell proliferation was significantly reduced and apoptosis significantly increased in the ALKBH5 + NC inhibitor group compared to the vector + NC inhibitor group ($P < 0.05$) (Figure 4A, B).

However, when miR-181b-5p expression was simultaneously inhibited along with ALKBH5 overexpression (ALKBH5 + miR-181b-5p inhibitor group), RA-FLS exhibited a marked increase in proliferation and a significant decrease in apoptosis compared to the ALKBH5 + NC inhibitor group ($P < 0.05$). These findings indicate that miR-181b-5p is a critical downstream effector of ALKBH5 in regulating RA-FLS proliferation and apoptosis.

Discussion

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by persistent synovial inflammation, joint pain, swelling, and progressive structural damage. Over time, the disease leads to joint deformity, disability, and a significant reduction in patients' quality of life. In this study, we investigated the role of ALKBH5, a known RNA demethylase, in the pathogenesis of RA, with a specific focus on its involvement in N6-methyladenosine (m6A) modification and the maturation of miR-181b-5p in fibroblast-like synoviocytes (FLS).

Our results demonstrate that ALKBH5 expression is significantly reduced in RA-FLS compared to normal FLS. Functionally, ALKBH5 overexpression inhibits RA-FLS proliferation and promotes apoptosis, whereas ALKBH5 knockdown has the opposite effects. Mechanistically, we found that ALKBH5 reduces m6A modification on pre-miR-181b-1, thereby enhancing its maturation to miR-181b-5p. This regulatory axis appears to be critical for modulating the pathogenic behavior of RA-FLS.

ALKBH5 belongs to the AlkB family of Fe(II)/ α -ketoglutarate-dependent

dioxygenases and functions primarily by demethylating m6A modifications on RNA^{20,21}. m6A is one of the most prevalent post-transcriptional modifications in eukaryotic mRNA and non-coding RNAs, playing essential roles in RNA metabolism, including splicing, stability, translation, and decay²². Recent evidence highlights m6A modifications as pivotal regulators of immune function, cell differentiation, and chronic inflammatory diseases such as RA²³.

Several studies have reported elevated m6A methylation and increased expression of METTL3—an m6A methyltransferase—in synovial tissues from RA patients²⁴. This upregulation likely stabilizes inflammation-related transcripts, thereby promoting synovial inflammation and joint destruction. Targeting the m6A modification machinery has emerged as a potential therapeutic strategy in RA. For instance, Xiao et al. demonstrated that ALKBH5 suppresses RA progression by demethylating and destabilizing NLRP3 mRNA via the ALKBH5–YTHDF2 axis, thus mitigating inflammation and joint damage²⁵.

Consistent with this literature, our study confirms that ALKBH5 expression is downregulated in RA-FLS. Restoration of ALKBH5 expression inhibits cell proliferation and enhances apoptosis, effects that are mediated at least in part by upregulating miR-181b-5p through m6A demethylation of its precursor, pre-miR-181b-1. These findings provide further evidence that ALKBH5 regulates RA pathogenesis through m6A-dependent post-transcriptional mechanisms.

Notably, our study identifies miR-181b-5p as a downstream effector of ALKBH5. While the role of miR-181b-5p in RA has not been fully elucidated, its dysregulation is implicated in several immune and inflammatory processes. Previous research in osteosarcoma demonstrated that ALKBH5-mediated demethylation of pre-miR-181b-1 promotes miR-181b-5p expression, which in turn inhibits YAP, a transcriptional regulator involved in cell proliferation and survival¹⁹. Although we did not assess YAP signaling in RA-FLS, it is plausible that a similar axis may exist in the RA context. Future investigations should explore the downstream targets of miR-181b-5p in RA, particularly those related to inflammatory and immune signaling.

Despite these promising findings, our study has certain limitations. First, all experiments were conducted *in vitro*, and the relevance of our results *in vivo* remains to be validated. Animal models or patient-derived organoid systems could help clarify the physiological role of the ALKBH5/miR-181b-5p axis in RA. Second, although we identified a novel regulatory mechanism involving ALKBH5 and miR-181b-5p, the broader network of m6A modification proteins and signaling pathways in RA remains incompletely understood. Further studies should examine the interaction of ALKBH5 with other m6A regulators and investigate additional non-coding RNA targets involved in RA progression.

Conclusion

ALKBH5 expression is significantly reduced in RA-FLS. Overexpression of ALKBH5 inhibits RA-FLS proliferation and promotes apoptosis by demethylating pre-miR-181b-1, thereby enhancing the maturation and expression of miR-181b-5p. These findings underscore the pivotal role of ALKBH5-mediated m6A demethylation in regulating RA-FLS behavior and suggest that targeting the ALKBH5/miR-181b-5p axis may represent a promising therapeutic approach for the treatment of RA.

Ethics approval

This study was approved by the Medical Ethics Committee of Ningbo Haishu District People's Hospital (Approval No. 2024015) and was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (1964) and its later amendments.

Consent to participate

Written informed consent was obtained from all participants prior to their inclusion in the study.

Authors' contributions

Dexin Wang and Dan Wang conceived and designed the study. Yu Fang, Rui Liu, and Wensuo Long were responsible for data acquisition. Huaiming Deng and Liwei Yu performed the data analysis. Dexin Wang and Dan Wang contributed to the manuscript revision. All authors read and approved the final version of the manuscript and are accountable for all aspects of the work.

Funding

This research is supported by the Zhejiang Province Medical and Health Science and Technology Plan Project (2023XYO24) and Key Discipline of Bone Surgery in Ningbo Haishu.

References

1. Xiao J, Cai X, Wang R, Zhou W, Ye Z. ALKBH5-YTHDF2 m6A modification axis inhibits rheumatoid arthritis progression by suppressing NLRP3. *Biochem Biophys Res Commun.* 2023;668:70–76.
2. Croia C, Bursi R, Suter D, Petrelli F, Alunno A, Puxeddu I. One year in review 2019: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol.* 2019;37(3):347–357.
3. Orange DE, Blachere NE, DiCarlo EF, Mirza S, Pannellini T, Jiang CS, et al. Rheumatoid arthritis morning stiffness is associated with synovial fibrin and neutrophils. *Arthritis Rheumatol.* 2020;72(4):557–564.
4. Hyltdgaard C, Hilberg O, Pedersen AB, Ulrichsen SP, Løkke A, Bendstrup E, et al. A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality. *Ann Rheum Dis.* 2017;76(10):1700–1706.
5. Li X, Qu M, Zhang J, Chen K, Ma X. CircASH2L facilitates tumor-like biologic behaviours and inflammation of fibroblast-like synoviocytes via miR-129-5p/HIPK2 axis in rheumatoid arthritis. *J Orthop Surg Res.*

- 2021;16(1):302.
6. Baito QN, Jaafar HM, Mohammad TAM. Piperine suppresses inflammatory fibroblast-like synoviocytes derived from rheumatoid arthritis patients via NF- κ B inhibition. *Cell Immunol.* 2023;391–392:104752.
 7. Falconer J, Murphy AN, Young SP, Clark AR, Tiziani S, Guma M, et al. Synovial cell metabolism and chronic inflammation in rheumatoid arthritis. *Arthritis Rheumatol.* 2018;70(7):984–999.
 8. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev.* 2010;233(1):233–255.
 9. Tsaltskan V, Firestein GS. Targeting fibroblast-like synoviocytes in rheumatoid arthritis. *Curr Opin Pharmacol.* 2022;67:102304.
 10. Liu L, Zhao H, Li J, Huang J, Xu Y, Li X, et al. ALKB homolog 5 (ALKBH5)-induced circPUM1 upregulation facilitated the progression of neuroblastoma via miR-423-5p/PA2G4 axis. *Pathol Res Pract.* 2023;248:154609.
 11. Liu Y, You Y, Lu Z, Yang J, Li P, Liu L, et al. N6-methyladenosine RNA modification-mediated cellular metabolism rewiring inhibits viral replication. *Science.* 2019;365(6458):1171–1176.
 12. Liu D, Li R, Xu S, Shi M, Kuang Y, Wang J, et al. SMOC2 promotes aggressive behavior of fibroblast-like synoviocytes in rheumatoid arthritis through transcriptional and post-transcriptional regulating MYO1C. *Cell Death Dis.* 2022;13(12):1035.
 13. Zhang Z, Zhou K, Han L, Small A, Xue J, Huang H, et al. RNA m6A reader YTHDF2 facilitates precursor miR-126 maturation to promote acute myeloid leukemia progression. *Genes Dis.* 2023;11(1):382–396.
 14. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell.* 2009;136(2):215–233.
 15. Wang Y, Zhang K, Yuan X, Xu N, Zhao S, Hou L, et al. miR-431-5p regulates cell proliferation and apoptosis in fibroblast-like synoviocytes in rheumatoid arthritis by targeting XIAP. *Arthritis Res Ther.* 2020;22(1):231.
 16. Wu J, Feng Z, Chen L, Li Y, Bian H, Geng J, et al. TNF antagonist sensitizes synovial fibroblasts to ferroptotic cell death in collagen-induced arthritis mouse models. *Nat Commun.* 2022;13(1):676.
 17. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. *Cold Spring Harb Protoc.* 2018;2018(6).
 18. Yuan Y, Yan G, He M, Lei H, Li L, Wang Y, et al. ALKBH5 suppresses tumor progression via an m6A-dependent epigenetic silencing of pre-miR-181b-1/YAP signaling axis in osteosarcoma. *Cell Death Dis.* 2021;12(1):60.
 19. Yue B, Song C, Yang L, Cui R, Cheng X, Zhang Z, et al. METTL3-mediated N6-methyladenosine modification is critical for epithelial-mesenchymal transition and metastasis of gastric cancer. *Mol Cancer.* 2019;18(1):142.
 20. Zhao Y, Hu J, Sun X, Yang K, Yang L, Kong L, et al. Loss of m6A demethylase ALKBH5 promotes post-ischemic angiogenesis via post-transcriptional stabilization of WNT5A. *Clin Transl Med.* 2021;11(5):e402.
 21. Chen J, Fang Y, Xu Y, Sun H. Role of m6A modification in female infertility and reproductive system diseases. *Int J Biol Sci.* 2022;18(9):3592–3604.
 22. Huang H, Weng H, Chen J. m6A modification in coding and non-coding RNAs: roles and therapeutic implications in cancer. *Cancer Cell.* 2020;37(3):270–288.
 23. Jiang X, Liu B, Nie Z, Duan L, Xiong Q, Jin Z, et al. The role of m6A modification in the biological functions and diseases. *Signal Transduct Target Ther.* 2021;6(1):74.
 24. Su Y, Wu Z, Liu Y, Liu X, Kang J, Jia J, et al. Increased m6A RNA methylation and METTL3 expression may contribute to the synovitis progression of rheumatoid arthritis. *Exp Cell Res.* 2024;442(2):114237.
 25. Xiao J, Cai X, Wang R, Zhou W, Ye Z. ALKBH5-YTHDF2 m6A modification axis inhibits rheumatoid arthritis progression by suppressing NLRP3. *Biochem Biophys Res Commun.* 2023;668:70–76.

Supplementary Table 1. Demographic and clinical features of patients with RA.

Age, yrs (mean±SD)	53.33±5.57
Sex [n (%)]	
Female	9 (75.00)
Male	3 (25.00)
Disease duration, yrs (mean±SD)	7.33±2.72
Rheumatoid factor-positive [n (%)]	8 (66.67)
DAS28 (CRP) (mean±SD)	5.03±0.31
Medication Status [n (%)]	
Methotrexate	9 (75.00)
Hydroxychloroquine	7 (58.33)
Sulfasalazine	5 (41.67)
Leflunomide	5 (41.67)
Tofacitinib	3 (25.00)
Baricitinib	1 (8.33)