MiR-221-5p is involved in the regulation of inflammatory responses in acute gouty arthritis by targeting IL-1β

Guangwen Li | Huihui Zhang | Hong Ma | Shiping Qu | Qian Xing | Ge Wang

Abstract

**Aim:** Gout is caused by the accumulation of deposited monosodium urate (MSU) crystals in the joints. Recent studies have shown that interleukin-1β (IL-1β) is a key inflammatory mediator of acute gouty arthritis (AGA), and its level is regulated by microRNAs (miRNAs). The purpose of this study was to study the role of miR-221-5p in the pathogenesis of AGA.

**Methods:** One hundred patients with AGA and 94 healthy individuals were recruited. The expression of serum miR-221-5p was determined by quantitative real-time polymerase chain reaction. The receiver operating curve (ROC) was applied for diagnostic value analysis. A luciferase reporter assay was performed to confirm the interaction of miRNA and the 3′-untranslated region (UTR) of IL-1β. Enzyme-linked immunosorbent assay was used to detect serum and proinflammatory factors.

**Results:** miR-221-5p had lower expression in the serum of AGA patients. The area under the curve was 0.884, the sensitivity was 82.0%, and the specificity was 80.9%. Serum miR-221-5p was negatively correlated with the expression levels of visual analog scale and IL-1β. Cell experiments showed that overexpression of miR-221-5p significantly inhibited the expression of inflammatory factors tumor necrosis factor-α, IL-8, and IL-1β, while down-regulation of miR-221-5p was the opposite. Luciferase analysis showed that IL-1β was the target gene of miR-221-5p.

**Conclusions:** This study confirmed that miR-221-5p regulates the production of inflammatory cytokines during the pathogenesis of AGA. These results suggested that miR-221-5p could be used as a potential therapeutic target for the treatment of AGA.

**Keywords**

acute gouty arthritis, IL-1β, inflammatory, MiR-221-5p, THP-1 cells

1 | INTRODUCTION

Gout attacks can be caused by hunger, trauma, surgery, ingestion of high-purine foods, excessive alcohol consumption, and medications that affect urate concentrations. It is divided into 3 clinical stages: acute gouty arthritis (AGA), intercritical gout, and chronic gout. AGA is an acute inflammation caused by the precipitation of urate crystals in joints. It is one of the most common types of auto-inflammatory arthritis, characterized by a sudden onset and significant pain that resolves spontaneously within a week. Interleukin-1β (IL-1β) is a central cytokine in the initiation of the acute inflammatory response, which plays a key role in the pathogenesis of gout, especially its role in the pathology of AGA. Tongfengshu capsule, a Chinese patent medicine, is composed of radix et rhizoma rhei palmati, semen plantaginis, rhizoma alismatis, radix achyranthis bidentatae and radix stephaniae tetrandrae;...
it has been used for the treatment of AGA with the involvement of IL-1β and tumor necrosis factor (TNF-α) regulation, suggesting the potential role of IL-1β and TNF-α in the progression of AGA. However, the mechanism of action of IL-1β in AGA is unclear.

MicroRNAs (miRNAs) are evolutionary conserved non-coding small RNA molecules that act as negative post-transcriptional gene regulators. Since a single miRNA molecule can target 100s of messenger RNAs (mRNAs), the abnormal expression of miRNA is related to the occurrence of many diseases. Recent research suggests that miRNAs may be involved in the development of arthritis. For example, the expression level of miR-155 in patients with gout arthritis is significantly higher than that in healthy individuals, and the overexpressed miR-155 can promote the production of monosodium urate (MSU)-induced inflammatory cytokines by reducing SHIP-1 levels.

Considering the important role of miRNAs in inflammatory diseases, especially AGA, more studies on miRNAs are urgently needed. MIR-221-5p has been widely reported to be aberrantly expressed in various metabolic diseases and involved in the diseases progression. For example, miR-221-5p is identified to be involved in the progression of diabetes. Another study also confirmed that miR-221-5p participates in the development of osteoarthritis. In addition, after constructing a miRNA gene pathway network, miR-221-5p is identified to be enriched in various metabolic pathways. Up to now, the molecular mechanism of miR-221-5p in AGA has been unclear.

In summary, miR-221-5p is critical for human cell inflammation and AGA. However, the functional role of miR-221-5p in AGA is not yet clear. Therefore, the purpose of this study was to study the role of miR-221-5p in the pathogenesis of AGA.

## 2 | MATERIALS AND METHODS

### 2.1 | Subject and sample collection

One hundred AGA patients and 94 healthy individuals matched in age and gender participated in the study. The 100 AGA patients recruited excluded the following conditions: (a) infection (b) tumor (c) rheumatoid arthritis. Five milliliters of peripheral blood samples were collected from each subject and immediately centrifuged. Subsequently, the serum samples were stored at −80°C for further analysis. All controls had no history of systemic inflammation or tumor. Clinical data including erythrocyte sedimentation rate (ESR), serum uric acid (SUA), visual analog scale (VAS), serum creatinine (SCR), age, gender, body mass index (BMI), leukocyte count, neutrophils count, and lymphocyte count were recorded in all participants.

The protocol of this study was approved by the Ethics Committee of Qingdao Municipal Hospital (no. 201710), and written informed consent was collected from each participant.

### 2.2 | Cell culture and transfection

The human monocyte THP-1 cell line was cultured in Roswell Park Memorial Institute 1640 medium (Life Technologies) and cultured in a 37°C, 5% CO₂ constant temperature incubator. THP-1 cells at 1.5 × 10⁵/mL were incubated in 96-well plates. The THP-1 cells were stimulated for 3 hours with 0.5 μmol/L phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) the day before stimulation. Then, the cells were stimulated with 250 μg/mL MSU crystals (Invitrogen) for 24 hours, causing inflammation, and presenting a variety of features of AGA. In order to regulate the expression level of miR-221-5p, cells were transfected with miR-221-5p mimic, miR-221-5p inhibitor, or their negative control (miR-NC), which was produced by Ribobio. Liposome 2000 (Invitrogen) was used for transfection according to the manufacturer’s instructions.

### 2.3 | Total RNA extraction and quantitative real-time polymerase chain reaction assay

Total RNA was extracted using TRIZOL reagent (Invitrogen). The miRNA bulge loop was reverse transcribed using the PrimeScript RT Reagent Kit (TaKaRa). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect gene expression using SYBR premix ExTaq M. II commercial kit (Takara) and the Applied Biosystems 7900 Real Time PCR System (Applied Biosystems). PCR parameters were as follows: 95°C for 3 minutes, followed by 40 cycles of 95°C for 10 seconds, 60°C for 20 seconds, and 72°C for 1 second. The relative gene expression was normalized to that of the internal control U6 according to the comparative delta CT (2^–ΔΔCt) method.

### 2.4 | Evaluation of inflammatory cytokines

The concentrations of IL-1β, IL-8, and TNF-α proteins in THP-1 cell culture supernatant were determined using an enzyme-linked immunosorbent assay kit (UK Abeam) in accordance with the manufacturer’s instructions. Each sample was analyzed 3 times.

### 2.5 | Luciferase reporter assay

The putative binding sites of miRNAs in the 3′-UTR (untranslated region) of the human IL-1β gene transcript were predicted by TargetScan (http://targetscan.org/), and then verified by luciferase reporter gene experiment. Cells were co-transfected with miR-221-5p mimic or inhibitor, and miR-221-5p wild type (WT) or mutant seed region (MUT) of IL-1β 3′-UTR. Lipofectamine 2000 (Invitrogen) was used for cell transfection. Relative luciferase activity was measured by the dual luciferase reporting system (Promega) according to the manufacturer’s instructions. The fluorescent activity of renal cells was used as an internal reference.

### 2.6 | Statistical analysis

In our study, all statistical analyses were performed with Prism 6 (GraphPad Software, San Diego, CA, USA) and IBM SPSS 20.
statistical software. The data were expressed as mean and standard deviation (SD). The differences between the 2 groups were compared by Student’s t test or one-way analysis of variance. Receiver operating characteristic (ROC) curves were used to determine the specificity and sensitivity of the diagnostic value of miR-221-5p for AGA. Correlation analysis was performed using Pearson correlation coefficients.

3 | RESULTS

3.1 | Clinical characteristics of different patient groups

Table 1 reports the main characteristics of the study population and laboratory results. A total of 194 individuals were included, the age range was 27-65 years. Among them, 94 subjects were healthy controls (44 males/50 females), and 100 AGA patients (48 males/52 females). There was no difference in age, gender distribution, BMI, ESR, and lymphocytes count between the groups (P > .05). There were significant differences in SUA, leukocyte count, neutrophil counts (P < .001). The VAS score in patients was 6.16 ± 2.36.

3.2 | The expression level of miR-221-5p and its correlation with VAS in AGA

We first studied the serum levels of miR-221-5p in healthy controls and AGA groups. The results of the study are shown in Figure 1A. The expression level of miR-221-5p in the serum of patients with AGA (0.61 ± 0.23) was significantly lower than that of the healthy control group (1.00 ± 0.25) (P < .05). The results indicated that miR-221-5p may be a key biomolecule for AGA and play an important biological role in its disease progression. In addition, in order to further explore the relationship between miR-221-5p and AGA, we also made a correlation between VAS and miR-221-5p. As shown in Figure 1B, serum miR-221-5p was negatively correlated to VAS (r = −.7671, P < .0001) in AGA patients. We concluded that miR-221-5p might be associated with the occurrence and severity of AGA.

3.3 | Diagnostic value of miR-221-5p in patients with AGA

Receiver operating curve curves were drawn based on the expression level of miR-221-5p in AGA patients and the control group to evaluate the diagnostic value of miR-221-5p in AGA patients. As shown in Figure 2, the miR-221-5p expression may be used to distinguish AGA patients from healthy individuals. The area under the curve (AUC) was 0.884, cut off value was 0.800, sensitivity was 82.0%, specificity was 80.9%. The results of this study confirmed the diagnostic value of miR-221-5p in differentiating AGA patients from healthy individuals.

3.4 | Effect of miR-221-5p on inflammatory responses in THP-1 cells

As shown in Figure 3A, the transfection of miR-221-5p mimic/inhibitor had a significant effect on the expression of miR-221-5p (P < .001). The expression levels of inflammatory factors significantly increased compared with the control group. Compared with the MSU group, the expression levels of TNF-α, IL-8, and IL-1β in the MSU group were significantly increased compared with the control group. Compared with the MSU group, the expression levels of inflammatory factors were significantly decreased in the miR-221-5p mimic transfection group (P < .001), and significantly increased in the miR-221-5p inhibitor group (P < .001).

3.5 | MiR-221-5p directly targets IL-1β in THP-1 cell

miRNAs were known to function by inhibiting the expression of their target genes. According to the Target scan analysis results, the binding sites of miR-221-5p in IL-1β are shown in Figure 4A(a). Luciferase reporter assay results showed that miR-221-5p mimic significantly inhibited luciferase activity of IL-1β WT 3′-UTR (Figure 4A(b), P < .001), while miR-221-5p inhibitor significantly increased luciferase activity. In addition, the luciferin activity of the mutant group was not affected by transfection with miR-221-5p mimic or miR-221-5p inhibitor. As shown in Figure 4B, the correlation between miR-221-5p expression and target gene IL-1β in AGA patients was also analyzed. The results showed that the expression of miR-221-5p in AGA patients was negatively correlated with IL-1β level (r = −.6762, P < .0001).

### TABLE 1 Baseline characteristics of the subjects

| Characteristics          | Controls (n = 94) | AGA (n = 100) | P value |
|--------------------------|------------------|---------------|---------|
| Age, y                   | 46.5 (11.8)      | 43.2 (11.7)   | .796    |
| Gender, male/female, n   | 44/50            | 48/52         | .868    |
| BMI, kg/m²               | 20.72 (1.73)     | 21.05 (1.64)  | .553    |
| ESR, mm/h                | 4.68 (2.81)      | 4.99 (3.01)   | .517    |
| SUA, μmol/L              | 185.15 (8.20)    | 227.44 (12.36)| <.001  |
| VSA, μmol/L              | —                | 6.16 (2.36)   |         |
| SCR, μmol/L              | 89.34 (26.48)    | 87.32 (26.27)| .6      |
| Leukocyte count, 10⁶/μL  | 7.06 (1.83)      | 25.92 (8.73)  | <.001   |
| Neutrophils count, 10³/μL| 4.49 (1.44)      | 31.19 (9.05)  | <.001   |
| Lymphocytes count, 10⁴/μL| 2.55 (0.94)      | 2.29 (0.86)   | .125    |

Note: Data are expressed as n or mean and standard deviation.
Abbreviations: AGA, acute gouty arthritis; BMI, body mass index; ESR, erythrocyte sedimentation rate; SCR, serum creatinine; SUA, serum uric acid; VSA, visual analog scale.
DISCUSSION

Gout is a common metabolic disease and AGA is one of the important complications. AGA is a group of clinical syndromes caused by MSU crystal deposition on bone, joints, and subcutaneous tissues, which is the most common initial symptom of gout. It is worth noting that while hyperuricemia has been classically associated with gouty arthritis, asymptomatic hyperuricemia is frequently found in metabolic syndrome, diabetes mellitus, chronic kidney disease, and osteoarthritis. Osteoarthritis is the most common form of arthritis overall, and gout and osteoarthritis frequently coexist in the same patient. Further, one study has confirmed the low expression of miR-221-5p in osteoarthritis. Therefore, its role in AGA attracts our interest. In our study, it was proposed that the expression of miR-221-5p in AGA patients was significantly lower than in healthy subjects, which was consistent with the results reported in osteoarthritis, indicating the association of miR-221-5p with AGA.

Serum miRNA is stable in stored samples, and it is more practical as a biomarker and easier to isolate than specific cell types of miRNAs, especially in AGA. Recent studies have suggested that miRNAs including miR-155 and miR-146a may be involved in the development of AGA in humans. For instance, a study has confirmed that miR-155 was upregulated in synovial fluid mononuclear cells (SFMCs) from patients with AGA. Another study confirmed that miR-146a plays a negative regulatory role in AGA in humans. However, the application of miR-221-5p in AGA has not been reported. In addition, a large number of studies have confirmed that VAS is an important indicator for the clinical assessment of AGA. Considering the dysregulation of miR-221-5p in AGA patients, we further studied the correlation between miR-221-5p and VAS, and the results showed that miR-221-5p was negatively correlated with VAS. Meanwhile, accurate and timely diagnosis and monitoring of treatment outcomes are critical to AGA patient prognosis. To solve this problem, reliable AGA biomarkers are urgently needed. Many studies have shown that miRNAs can be a biomarker useful for diagnosis and prognosis. In view of the significant correlation between miR-22-5p and VAS, we further evaluated the ability of serum miR-221-5p to differentiate AGA from healthy individuals by establishing ROC curves. High sensitivity and specificity of AUC was detected, demonstrating that miR-221-5p had the ability to distinguish AGA patients from healthy controls.

Acute gouty arthritis is one of the most painful inflammatory conditions. Therefore, the onset of AGA is accompanied by all the characteristics of an acute inflammatory response. These include intimal hyperplasia, infiltration of neutrophils, mononuclear phagocytes, and lymphocytes. miRNAs are confirmed to be central players in pathways associated with MSU-induced inflammation and gouty arthritis. Further, another study has confirmed that miRNA inhibited the expression of IL-1β induced by MSU in THP-1 cells, such as miR-488 and miR-920. In the present study, THP-1 cells were stimulated with MSU crystals to mimic inflammation features of AGA, and the cell experiments demonstrated that miR-221-5p overexpression inhibited the release of inflammatory cytokines, including IL-1β, IL-8 and TNF-α. The abnormal expression of miRNAs can affect specific targets and pathways, leading to the phenotype of auto-inflammatory disease, which is also supported by some in vivo studies. For example, the researchers have found that miR-488 and miR-920 can directly target the 3’-UTR of IL-1β in gouty arthritis. It is demonstrated that IL-1β can be involved in the
The identification of miRNAs possibly targeting IL-1β in gouty arthritis is another major goal for the future. Therefore, targeting miRNAs may be an effective option for treating auto-inflammatory disease in the future. In our results, the luciferase reporter assay analysis showed that IL-1β may be involved in the AGA process as a target gene of miR-221-5p. All evidence supported our conclusion that miR-221-5p might be involved in the development of AGA and inhibit inflammatory responses via targeting IL-1β. Further, the luciferin activity of the mutant type group was not affected by the transfection of miR-221-5p mimic or miR-221-5p inhibitor. **P < .001. B, Correlations of miR-221-5p expression with the IL-1β expression (r = −0.6762, P < .0001) in AGA patients. In addition, the study population should be expanded to better verify the current study effect.

5 | CONCLUSION

It is commonly known that auto-inflammation plays a pivotal role in the pathology of AGA. However, the exact etiology and pathogenesis are poorly understood. In general, we found that miR-221-5p was downregulated in patients with AGA. ROC curves were drawn based on the expression level of miR-221-5p and the diagnostic value of miR-221-5p in distinguishing AGA patients from healthy people was...
confirmed. Therefore, it has potential as a therapeutic or biomarker for AGA. miR-221-5p may participate in the development of AGA patients by acting on target gene IL-1β to inhibit the release of inflammatory factors in THP-1 cells.

CONFLICT OF INTEREST
The authors have declared that no conflict of interest exists.

ORCID
Ge Wang https://orcid.org/0000-0001-5222-9403

REFERENCES
1. Ene-Stoescu D, Gorbien MJ. Gouty arthritis. A primer on late-onset gout. *Geriatrics*. 2005;60(7):24-31.
2. Jacobs CL, Stern PJ. An unusual case of gout in the wrist: the importance of monitoring medication dosage and interaction. A case report. *Chiropr Osteopat*. 2007;15:16.
3. Dalbeth N, Merriman TR, Stamp LK. Gout. *Lancet*. 2016;388(10055):2039-2052.
4. Neogi T. Clinical practice. *N Engl J Med*. 2011;364(5):443-452.
5. Gong QY, Chen Y. Correlation between P2X7 receptor gene polymorphisms and gout. *Rheumatol Int*. 2015;35(8):1307-1310.
6. Chi X, Zhang H, Zhang S, Ma K. Chinese herbal medicine for gout: a review of the clinical evidence and pharmacological mechanisms. *Chin Med*. 2020;15:17.
7. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. *Nature*. 2008;455(7209):64-71.
8. Jiang L, Huang J, Chen Y, et al. Identification of several circulating microRNAs from a genome-wide circulating microRNA expression profile as potential biomarkers for impaired glucose metabolism in polycystic ovarian syndrome. *Endocrine*. 2016;53(1):280-290.
9. Jin HM, Kim TJ, Choi JH, et al. MicroRNA-155 as a proinflammatory regulator via SHIP-1 down-regulation in acute gouty arthritis. *Arthritis Res Ther*. 2014;16(2):R88.
10. Dalbeth N, Pool B, Shaw OM, et al. Role of miR-146a in regulation of the acute inflammatory response to monosodium urate crystals. *Ann Rheum Dis*. 2015;74(4):786-790.
11. Lyons JG, Lobo E, Martorana AM, Myerscough MR. Clonal diversity in carcinomas: its implications for tumour progression and the contribution made to it by epithelial-mesenchymal transitions. *Clin Exp Metastasis*. 2008;25(6):665-677.
12. Liu HN, Li X, Wu N, et al. Serum microRNA-221 as a biomarker for diabetic retinopathy in patients associated with type 2 diabetes. *Int J Ophthalmol*. 2018;11(12):1889-1897.
13. Zhang X, Shang L, Shang J, et al. Combined microRNAome and transcriptome analysis of follicular phase and luteal phase in porcine ovaries. *Reprod Domest Anim*. 2019;54(7):1018-1025.
14. Yuan X, Fan YS, Xu L, Xie GQ, Feng XH, Qian K. Jia-Wei-Si-Miao-Wan alleviates acute gouty arthritis by targeting NLRP3 inflammasome. *J Biol Regul Homeost Agents*. 2019;33(1):63-71.
15. Zhou M, Ze K, Wang Y, et al. Huzhang Tongfeng granule improves monosodium urate-induced inflammation of gouty arthritis rat model by downregulation of Cyr61 and related cytokines. *Evid Based Complement Alternat Med*. 2020:2020:e9238797.
16. Albu A, Para I, Porojan M. Uric acid and arterial stiffness. *Ther Clin Risk Manag*. 2020;16:39-54.
17. Papanagou P, Stivarou T, Tsironi M. The role of miRNAs in common inflammatory arthropathies: osteoarthritis and gouty arthritis. *Biomolecules*. 2016;6(4):44.
18. Neogi T, Krasnokutsky S, Pillinger MH. Urate and osteoarthritis: evidence for a reciprocal relationship. *Joint Bone Spine*. 2019;86(5):576-582.
19. Ormseth MJ, Solus JF, Sheng Q, et al. Development and validation of a MicroRNA panel to differentiate between patients with rheumatoid arthritis or systemic lupus erythematosus and controls. *J Rheumatol*. 2020;47(2):188-196.
20. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA*. 2008;105(30):10513-10518.
21. Yang Q, Zhang Q, Qing Y, Zhou L, Mi Q, Zhou J. miR-155 is dispensable in monosodium urate-induced gouty inflammation in mice. *Arthritis Res Ther*. 2018;20(1):144.
22. Zhang QB, Qing YF, Yin CC, et al. Mice with miR-146a deficiency develop severe gouty arthritis via dysregulation of TRAF 6, IRAK 1 and NALP3 inflammasome. *Arthritis Res Ther*. 2018;20(1):45.
23. Jena M, Tripathy A, Mishra A, Maiti R. Effect of canakinumab on clinical and biochemical parameters in acute gouty arthritis: a meta-analysis. *Inflammopharmacology*. 2020.
24. Sun X, Zhou X, Zhang Y, Zhu X, Liu H. Systematic review and meta-analysis of diagnostic accuracy of miRNAs in patients with pancreatic cancer. *Dis Markers*. 2018;2018:e6292396.
25. Chai W, Tai Y, Shao X, et al. Electroacupuncture alleviates pain responses and inflammation in a rat model of acute gout arthritis. *Evid Based Complement Alternat Med*. 2018;2018:e2598975.
26. Zhou W, Wang Y, Wu R, He Y, Su Q, Shi G. MicroRNA-488 and -920 regulate the production of proinflammatory cytokines in acute gouty arthritis. *Arthritis Res Ther*. 2017;19(1):203.
27. Liu CW, Sung HC, Lin SR, et al. Resveratrol attenuates ICAM-1 expression and monocyte adhesiveness to TNF-alpha-treated endothelial cells: evidence for an anti-inflammatory cascade mediated by the miR-221/222/AMPK/p38/NF-kappaB pathway. *Sci Rep*. 2017;7:e44689.
28. Ceribelli A, Sato Y, Chan EK. MicroRNAs and autoimmunity. *Curr Opin Immunol*. 2012;24(6):686-691.

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