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ORIGINAL ARTICLE

Analysis of LINC00472 as a biomarker of osteoarthritis and its clinical value

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ABSTRACT

Objectives: This study aimed to investigate the effect of LINC00472 in osteoarthritis (OA) and its molecular mechanism.

Patients and methods: This prospective study was conducted with 110 patients (59 females, 51 males; mean age: 58.6±10.3 years; range, 37 to 79 year) with OA and 101 healthy controls (58 females, 43 males; mean age: 60.6±10.3 years; range, 35 to 78 years) between June 2020 and November 2022. First, we measured LINC00472 levels in OA patients using RT-qPCR (real-time quantitative reverse transcription polymerase chain reaction). Afterward, we treated human chondrocytes with interleukin (IL)-1β, which aimed to construct an OA cellular model to explore the function of LINC00472 in OA. Messenger RNA levels were detected by RT-qPCR. Apoptosis was measured by flow cytometry. Cell viability was measured by CCK-8 (cell counting kit-8) assay. Enzyme-linked immunosorbent assay was used to detect inflammatory factor levels. Finally, we verified the targeting of miR-361-5p with LINC00472 and MECP2 by luciferase assay and RNA immunoprecipitation.

Results: In OA patients and OA cells, LINC00472 and MECP2 levels were increased, and miR-361-5p levels were decreased. LINC00472 levels were negatively correlated with miR-361-5p levels and positively correlated with MECP2 levels. In human chondrocytes, LINC00472 knockdown inhibited apoptosis, cellular inflammation, and extracellular matrix degradation. However, miR-361-5p inhibitor reversed these effects. In addition, LINC00472 knockdown downregulated MECP2 levels, and miR-361-5p inhibitor reversed the effect.

Conclusion: LINC00472 is involved in chondrocyte apoptosis, extracellular matrix degradation, and cellular inflammation in OA through the miR-361-5p/MECP2 axis. LINC00472 may regulate OA development by increasing MECP2 expression through sponged miR-361-5p and may be a new target for OA diagnosis and treatment.

Keywords: Chondrocytes, LINC00472, miR-361-5p, MECP2, osteoarthritis.

Osteoarthritis (OA) is a common chronic degenerative disease. The main pathophysiologic features of OA are the destruction of articular cartilage, synovial inflammation, and subchondral osteosclerosis.[1] Inflammatory response plays an important role in the progression of OA. Inflammatory factors such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6 cause chondrocyte apoptosis and extracellular matrix (ECM) degradation.[2,3] Chondrocytes and ECM are the main components of articular cartilage, and chondrocyte apoptosis, fibrosis, and abnormal ECM metabolism all contribute to the development of OA.[4] Worldwide, about 14% of people over the age of 60 are diagnosed with OA, and its prevalence increases with age.[5] Worldwide, OA is estimated to cause more than $300 billion in lost income and healthcare costs annually.[6] Due to its high prevalence and substantial costs, OA has severely reduced the quality of life for many individuals and has become a burden on the healthcare system.[7] Currently, OA is diagnosed by radiology and physical examination. However, in the early stages of OA, it is difficult to diagnose by radiology and physical examination, and the sensitivity of radiography to monitor disease progression is poor.[8] As research advances, biomarkers are coming to the forefront. Compared to radiology, biomarkers can dynamically respond to disease progression and treatment efficacy.[9] An increasing number of biomarkers are being used for diagnosis and

prognosis, which also provide new research targets for disease treatment.

Studies have shown that long noncoding RNAs (lncRNAs) are involved in many biological processes (immunity, cell proliferation, apoptosis, and tumor cell migration),[10-12] and variety of diseases are associated with aberrant expression of lncRNA. Research has shown that lncRNAs regulate the development of OA. MALAT1 exacerbates OA by sponging miR-150-5p.[13] SNHG12 is upregulated in tissues with OA, and the upregulation of the lncRNA SNHG12 protects chondrocytes by inhibiting inflammation and ECM degradation.[14] The lncRNA THUMPD3-AS1 was shown to enhance the inflammatory response of OA.[15] Hua et al.[16] analyzed the GSE126963 dataset on OA samples and identified 17 lncRNAs with upregulated expression, including LINC00472. LINC00472 exerted proinflammatory effects in a variety of inflammatory models. In a sepsis-induced liver injury model, LINC00472 exacerbated liver injury by modulating the levels of inflammatory factors.[17] In addition, downregulation of LINC00472 inhibited neuropathic pain by decreasing the expression of proinflammatory factors such as IL-1β, TNF-α, and IL-6.[18] However, LINC00472 has not been reported in OA, and the function and molecular mechanism of LINC00472 in OA remains unclear. Therefore, based on the mentioned studies, we hypothesized that LINC00472 may be involved in the occurrence and development of OA and may be associated with chondrocyte proliferation and apoptosis, ECM degradation, and inflammation. Consequently, this study aimed to provide new research ideas and targets for the diagnosis and treatment of OA.

PATIENTS AND METHODS

This prospective study was conducted with 110 patients (59 females, 51 males; mean age: 58.6±10.3 years; range, 37 to 79 year) with OA and 101 healthy controls (58 females, 43 males; mean age: 60.6±10.3 years; range, 35 to 78 years) at the Xuzhou No.1 People's Hospital, the Affiliated Xuzhou Municipal Hospital of Xuzhou Medical University, Department of Orthopaedics between June 2020 and November 2022. We collected 110 cartilage tissues from OA patients who underwent total knee replacement and 101 normal cartilage tissues from amputees without a history of OA or acute gouty arthritis. After the cartilage tissue was separated, it was carefully stored at −80°C. The inclusion criteria were as follows: *(i)* recurrent knee pain within the last month; *(ii)* narrowing of the articular space, subchondral osteosclerosis, or capsular degeneration on standing radiographs; *(iii)* age ≥50 years; *(iv)* duration of morning stiffness ≤30 min; *(v)* bone friction sound/friction sensation during activity. The diagnosis was confirmed if the first criterion and any other two criteria were met. The inclusion criteria for OA patients were as follows: *(i)* meeting the diagnostic criteria; *(ii)* age of 40 to 75 years; *(iii)* knee OA graded by Kellgren-Lawrence classification as II-IV; *(iv)* meeting the indications of total knee arthroplasty. The exclusion criteria were as follows: *(i)* patients with malignant tumors, fractures, or traumatic injuries; *(ii)* patients with cardiovascular and cerebrovascular diseases, gastric and duodenal ulcers, and diseases of liver and kidney; *(iii)* patients with psychiatric disorders; *(iv)* patients with gout, inflammatory arthritis, and allergy. The study protocol was approved by the Xuzhou No.1 People's Hospital Ethics Committee (date: on 25.02.2020, no. 2020125). Written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

WOMAC score

The Western Ontario and McMaster Universities Arthritis Index (WOMAC) is often used as a tool to assess the severity of arthritis and the efficacy of treatment. WOMAC evaluates the structure and function of joints in terms of pain, stiffness, and joint function and consists of 24 items that encompass the basic signs and symptoms of OA. Each item is scored on a scale of 0 to 4, with 0 indicating no pain, no stiffness, and no dysfunction, and 4 indicating pain, stiffness, and severe dysfunction. The total score is between 0 and 96, with higher scores indicating greater disease severity.

Cell culture and treatment

Human chondrocytes were purchased from Pricella Biotech (Wuhan Pricella Biotechnology Co., Ltd., Wuhan, China) and were cultured in a complete culture medium

(Wuhan Pricella Biotechnology Co., Ltd., Wuhan, China) for human chondrocytes. To construct OA cell model *in vitro*, human chondrocytes with different concentrations of recombinant human IL-1β protein (0, 2.5, 5, and 10 ng/mL) were treated for 24 h.

Cell culture and transfection

si-NC, si-LINC00472, NC inhibitor, and miR-361-5p inhibitor were purchased from GenePharma (Shanghai GenePharma Co., Ltd, Shanghai, China). Subsequently, we used Lipofectamine 293 (Beyotime Biotech Inc., Shanghai, China) to transfect the above substances into chondrocytes for follow-up studies. The transfection time was 24 h, and the cell density reached at least 70% before transfection.

Cell viability assay

Chondrocytes at the logarithmic growth stage were collected, and cell suspension concentrations were adjusted. A total of 3×10^3 cells were seeded per well in a 96-well plate in a volume of 100 µL. After 24 h of incubation, 10 µL of CCK-8 (cell counting kit-8) solution was added to each well, and the absorbance was measured at 450 nm by a microplate reader.

Cell apoptosis assay

Annexin V-APC/PI Apoptosis Kit (Elabscience Biotechnology Co., Ltd, Wuhan, China) and flow cytometry were used to detect chondrocyte apoptosis. First, 1×10^5 cells were washed twice with phosphate-buffered saline. Second, 5 µL of the Annexin V-APC reagent and 5 µL of the PI reagent were added to the cell suspension and gently mixed. Finally, the mixture was incubated for 15 to 20 min in the dark and then measured by flow cytometry.

Enzyme-linked immunosorbent assay

IL-6 and IL-8 levels were examined using commercial human IL-6 and IL-8 enzyme-linked immunosorbent assay (ELISA) kits (Solarbio, Beijing, China), with strict adherence to the instructions of the kit.

RNA extraction and real-time quantitative reverse transcription polymerase chain reaction

Total RNA was obtained from cartilage tissues and chondrocytes by using the TRIzol reagent (Invitrogen, Waltham, MA, USA). Afterward, we reversed RNA into cDNA (complementary DNA) by using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara Biotechnology, Dalian, Liaoning, China). Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) amplification reactions were performed after mixing cDNA, primers, RNase-free water, and the SYBR Green Quantitative RT-qPCR Kit (Sigma-Aldrich, St. Louis, Missouri, USA) reagent well. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was the reference gene. Relative expression was calculated by the 2-ΔΔCT method.

To explore the distribution of LINC00472 in chondrocytes, we used the PARIS Kit (Invitrogen, Waltham, MA, USA) to isolate RNA from the nucleus and cytoplasm of chondrocytes. We performed RT-qPCR to measure LINC00472 levels in the nucleus and cytoplasm. U6 and GAPDH were employed as nuclear or cytoplasmic controls.

RNA immunoprecipitation assay

First, chondrocytes were lysed in RNA immunoprecipitation (RIP) buffer. Secondly, the cell lysate was incubated with magnetic beads containing Ago2 antibodies or IgG antibodies. Finally, we added proteinase K to the sample to remove the protein and obtain purified RNA. Total RNA was analyzed by RT-qPCR.

Dual-luciferase reporter assay experiment

LINC00472-WT, LINC00472-MUT, methyl-CpG-binding protein 2 (MECP2)-WT, and MECP2-MUT sequences were synthesized by GenePharma (Shanghai GenePharma Co., Ltd, Shanghai, China). Then, we inserted the sequence mentioned above into pmirGLO reporter vectors to obtain the LINC00472-WT vector, LINC00472-MUT vector, MECP2-WT vector, and MECP2-MUT vector. Chondrocytes were co-transfected with LINC00472-WT vector, LINC00472-MUT vector, MECP2-WT vector or MECP2-MUT vector, and NC inhibitor or miR-361-5p inhibitor. After 48 h, luciferase activity was measured.

Bioinformatic analysis

The ENCORI, lncRNASNP2, and DIANA databases predicted target micro RNAs (miRNAs) for LINC00472. Furthermore, the targets of

miR-361-5p were predicted in miRDB, miRWalk, Tarbase, miRPathDB, and TargetScan online software. Venn diagrams were created by the web tool to examine the overlapping miRNAs or target genes. To calculate the protein-protein interaction (PPI) networks of overlapping targets by the STRING database, proteins in the PPI network of nodes were mapped to their original genes. From this point forward, PPI models were referred to as "genes."

Statistical analysis

The statistical power was calculated using the G*power software version 3.1.9.4 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). A total sample size of 211 individuals provided a statistical power of 99% with a large effect size of 0.5 and an alpha error probability of 0.05.

Data were analyzed using IBM SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9.0 softwares (GraphPad Software Inc., La Jolla, CA, USA) were used for statistical analysis and charting. Normality tests were performed using the Kolmogorov-Smirnov test. For normally distributed variables, data were presented as mean ± standard deviation (SD) and analyzed by Student's t-test and one-way analysis of variance. For nonnormally distributed variables, data were presented as median (interquartile range) and tested by the nonparametric two-independent-sample Kolmogorov-Smirnov test. Continuous variables were analyzed by the chi-square test and presented as frequency and percentage. Each experiment was repeated three times with standardized experimental equipment and procedures to minimize experimental error. A p-value <0.05 was considered statistically significant.

RESULTS

As shown in Table 1, no statistical differences were observed in age, sex, body mass index, smoking, and alcohol consumption between the two groups (p>0.05). The WOMAC score in OA patients was 10.00 (7.00, 14.25).

LINC00472 as a diagnostic biomarker for OA

Compared to healthy controls, LINC00472 levels were raised in OA patients (p<0.0001, Figure 1a). LINC00472 levels were positively related to WOMAC scores (p<0.0001, R=0.793, Figure 1b). As shown in Figure 1c, the area under the curve was 0.824, with a sensitivity of 74.55% and specificity of 78.85%, demonstrating that LINC00472 could accurately identify OA from healthy individuals.

Silencing LINC00472 reversed IL-1β-induced chondrocyte damage

Interleukin-1β could induce the high expression of LINC00472 in chondrocytes, and LINC00472 levels gradually increased with the increase of IL-1β concentration and the prolongation of IL-1β induction time (Figures 2a, b). To investigate the function of LINC00472 in OA, we constructed and used the silenced RNA of LINC00472. As shown in Figure 3a, IL-1β induced the expression of

Table 1. Clinical characteristics of healthy controls and patients with OA

	OA patients (n=110)					Healthy individuals (n=104)					
Parameters	n	%	Mean±SD	Median	IQR	n	%	Mean±SD	Median	IQR	*p*
Age (year)			58.6±10.3					60.6±10.3			0.153
Sex Female	59	53.64				58	55.77				0.785
BMI (kg/m^2)			22.61±3.68					22.19±4.22			0.434
Smoke	57	51.82				59	56.73				0.495
Drinking	62	56.36				53	50.96				0.493
WOMAC score				10.00	7.00-14.25				-	-	-

OA: Osteoarthritis; SD: Standard deviation; IQR: Interquartile range; WOMAC: Western Ontario and McMaster Universities Arthritis Index.

LINC00472 in chondrocytes, and the level of LINC00472 did not change significantly after transfection of chondrocytes with si-NC. However, silencing LINC00472 reduced IL-1β-induced expression of LINC00472, demonstrating that the cell transfer in this study was effective. Furthermore, IL-1β inhibited chondrocyte activity and promoted apoptosis, and silencing LINC00472 partially reversed these effects (Figures 3b, c). Downregulation of LINC00472 increased COL2A1 and aggrecan levels and decreased ADAMTS4 and MMP13 levels in chondrocytes (Figure 3d). In addition, low expression of LINC00472 downregulated levels of inflammatory factors (Figure 3e).

LINC00472 sponged miR-361-5p

According to subcellular localization experiments, LINC00472 was mainly expressed in the cytoplasm (Figure 4a), which suggests that LINC00472 may be a sponge of miRNA. Next, we obtained four overlapping target miRNA of LINC00472 from ENCORI, lncRNASNP2, and DIANA databases: miR-361-5p, miR-196a-5p, miR-378a-3p, and miR-378c (Figure 4b). As shown in Figure 4c, there were complementary binding sites between LINC00472 and miR-361-5p. Both LINC00472 and miR-361-5p could be enriched in Ago2 (Figure 4e). After transfection with miR-361-5p inhibitor, the luciferase activity of LINC00472-WT was increased, but that of LINC00472-MUT was not affected by miR-361-5p inhibitor (Figure 4d). Compared to healthy controls, miR-361-5p levels were lower in OA patients, and miR-361-5p levels were negatively correlated with LINC00472 levels (Figure 4f, g). The miR-361-5p levels decreased with the increase of IL-1β concentration and the extension of treatment time. However, after downregulating LINC00472, miR-361-5p levels in chondrocytes increased significantly (Figures 4h-j).

LINC00472 sponged miR-361-5p in the regulation of chondrocyte injury

The miR-361-5p messenger RNA levels were increased in si-LINC00472-transfected chondrocytes and decreased in si-LINC00472 co-transfected chondrocytes with miR-361-5p inhibitor (Figure 5a). Downregulation of LINC00472 increased chondrocyte viability

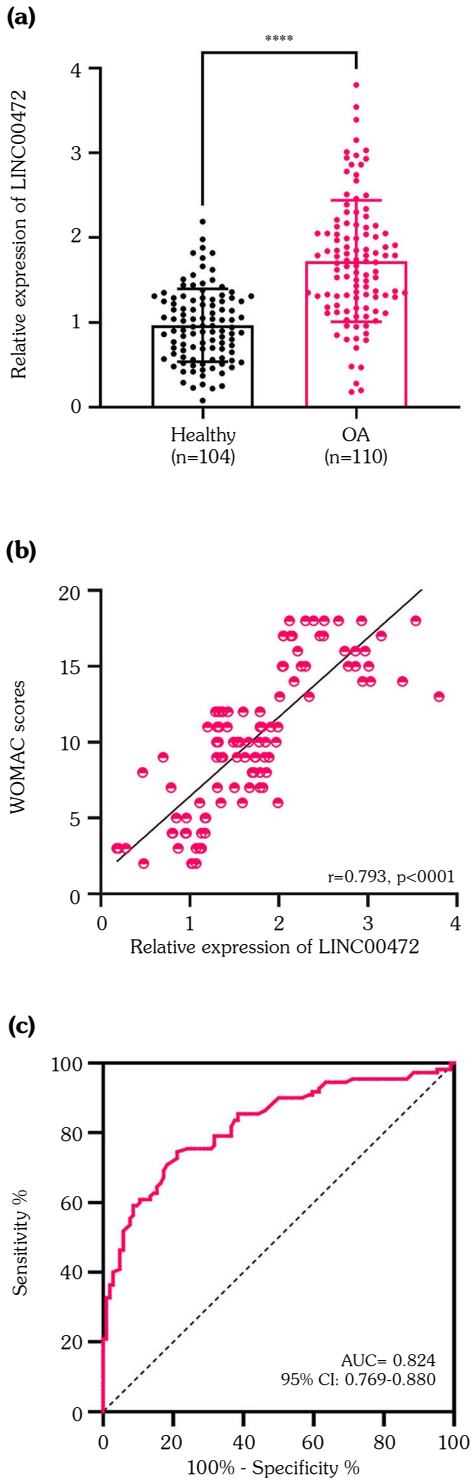

Figure 1. LINC00472 distinguished OA patients from controls. **(a)** LINC00472 levels were determined by RT-qPCR. **(b)** WOMAC score was positively correlated with LINC00472 levels. **(c)** The performance of LINC00472 in distinguishing OA patients from controls was analyzed by a receiver operating characteristic curve.

OA: Osteoarthritis; WOMAC: Western Ontario and McMaster Universities Arthritis Index; **** p<0.0001.

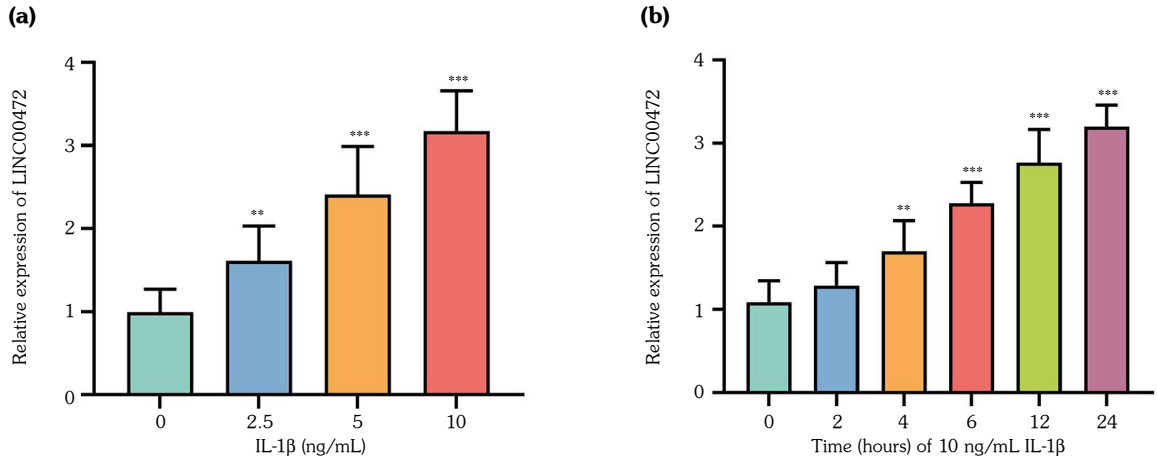

Figure 2. LINC00472 levels after IL-1β treatment of chondrocytes. **(a)** LINC00472 levels at different IL-1β concentrations. **(b)** LINC00472 levels under different IL-1β treatment times.

IL: Interleukin; ** p<0.01; *** p<0.001.

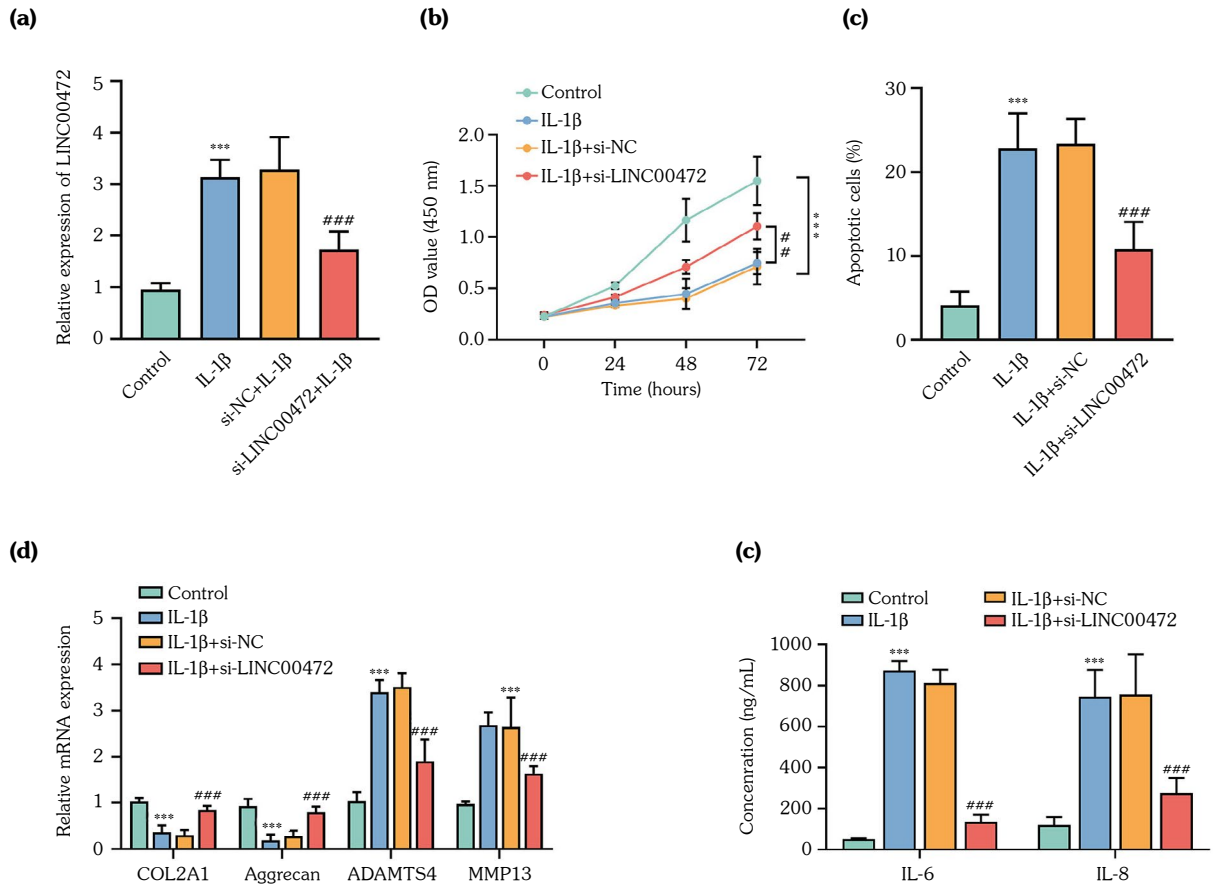

Figure 3. LINC00472 knockdown restrained chondrocyte damage induced by IL-1β. **(a-e)** After transfection with si-NC or si-LINC00472, chondrocytes were treated with 10 ng/mL IL-1β. **(a)** LINC00472 levels were determined by RT-qPCR. **(b)** Differences in cell viability between different groups. CCK-8 assay was used to determine cell viability. **(c)** Differences in apoptosis between different groups. Flow cytometry was used to detect apoptosis. **(d)** The level of factors associated with ECM degradation was determined by RT-qPCR. **(e)** The concentrations of IL-6 and IL-8 were determined by ELISA.

IL: Interleukin; RT-qPCR: Real-time quantitative reverse transcription polymerase chain reaction; ECM: Extracellular matrix; *** p<0.001 *vs.* Control; ### p<0.001 *vs.* IL-1β.

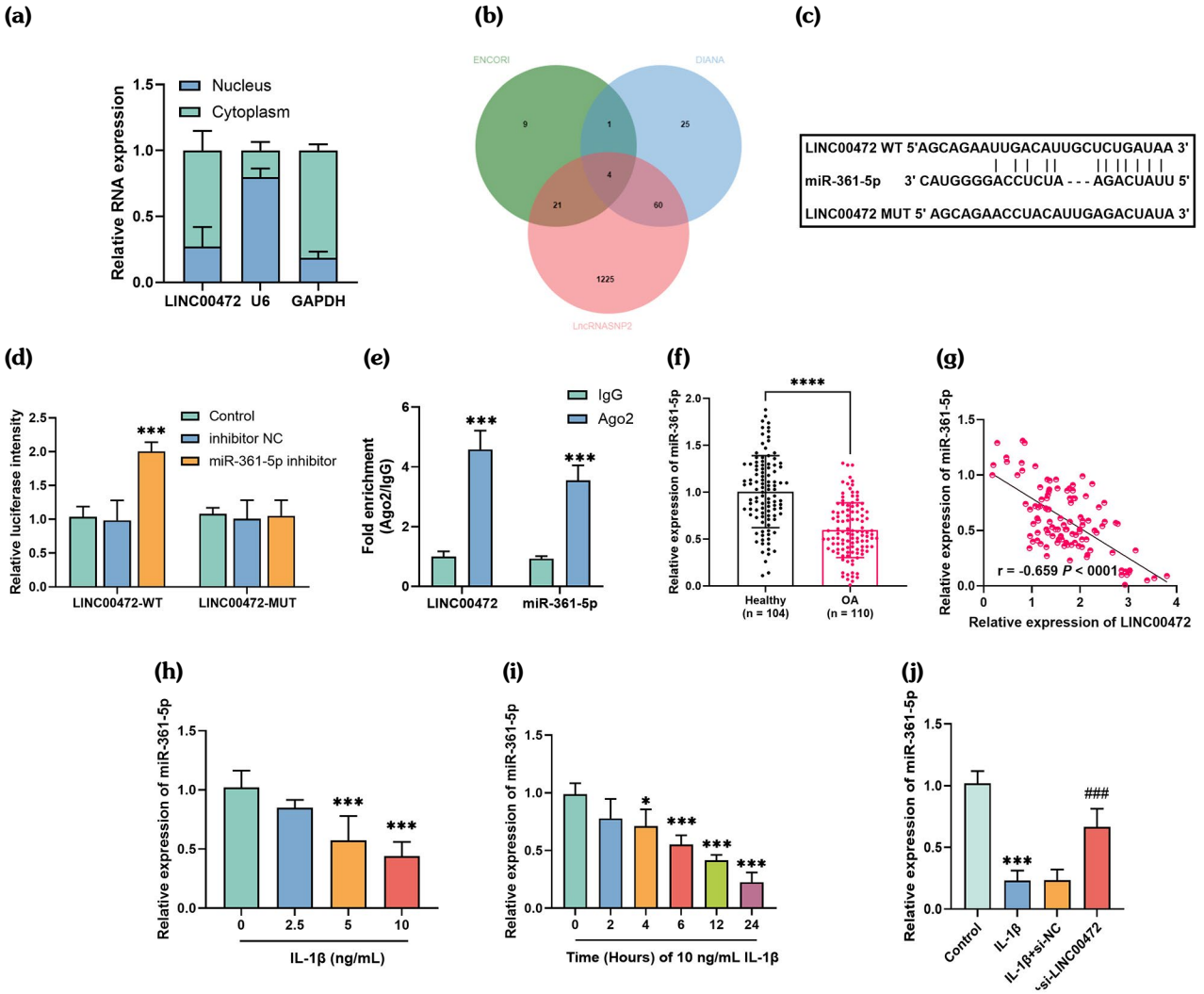

Figure 4. LINC00472 sponged miR-361-5p. **(a)** Subcellular localization analysis of LINC00472 in chondrocytes. **(b)** The overlapping target miRNA for LINC00472. **(c)** Binding site of LINC00472 to miR-361-5p and mutation site of LINC00472. **(d, e)** Dual luciferase analysis experiments and RIP analysis verified the relationship between LINC00472 and miR-361-5p. **(f)** miR-361-5p levels were measured by RT-qPCR. **(g)** The correlation between LINC00472 and miR-361-5p levels was analyzed by Pearson correlation analysis. **(h, i)** miR-361-5p levels at different IL-1β concentrations and different treatment times. **(j)** After transfection with si-NC or si-LINC00472, chondrocytes were treated with 10 ng/mL IL-1β. miR-361-5p levels were determined by RT-qPCR.

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; IgG: Immunoglobulin G; IL: Interleukin; miRNA: Micro ribonucleic acid; RT-qPCR: Real-time quantitative reverse transcription polymerase chain reaction; * p<0.05; *** p<0.001; **** p<0.0001 *vs.* Control; ### p<0.001 *vs.* IL-1β.

and inhibited apoptosis and ECM degradation, while miR-361-5p inhibitor reversed these results (Figures 5b-d). The miR-361-5p inhibitor also overruled the regulatory effect of si-LINC00472 on inflammation factor (Figure 5e).

MECP2 was the target of miR-361-5p

The targets of miR-361-5p were predicted in miRDB, miRWalk, Tarbase, miRPathDB, and TargetScan online software. It found a total of 67 overlapping targets (Figure 6a). The PPI network contained 67 nodes and 54 edges, and the PPI enrichment p-value was 0.0051. The top 10 genes in the PPI network for degree nodes included MECP2 (Figure 6b). As shown in Figure 6c, complementary sites existed between miR-361-5p and MECP2. After transfection with miR-361-5p inhibitor, the luciferase activity of

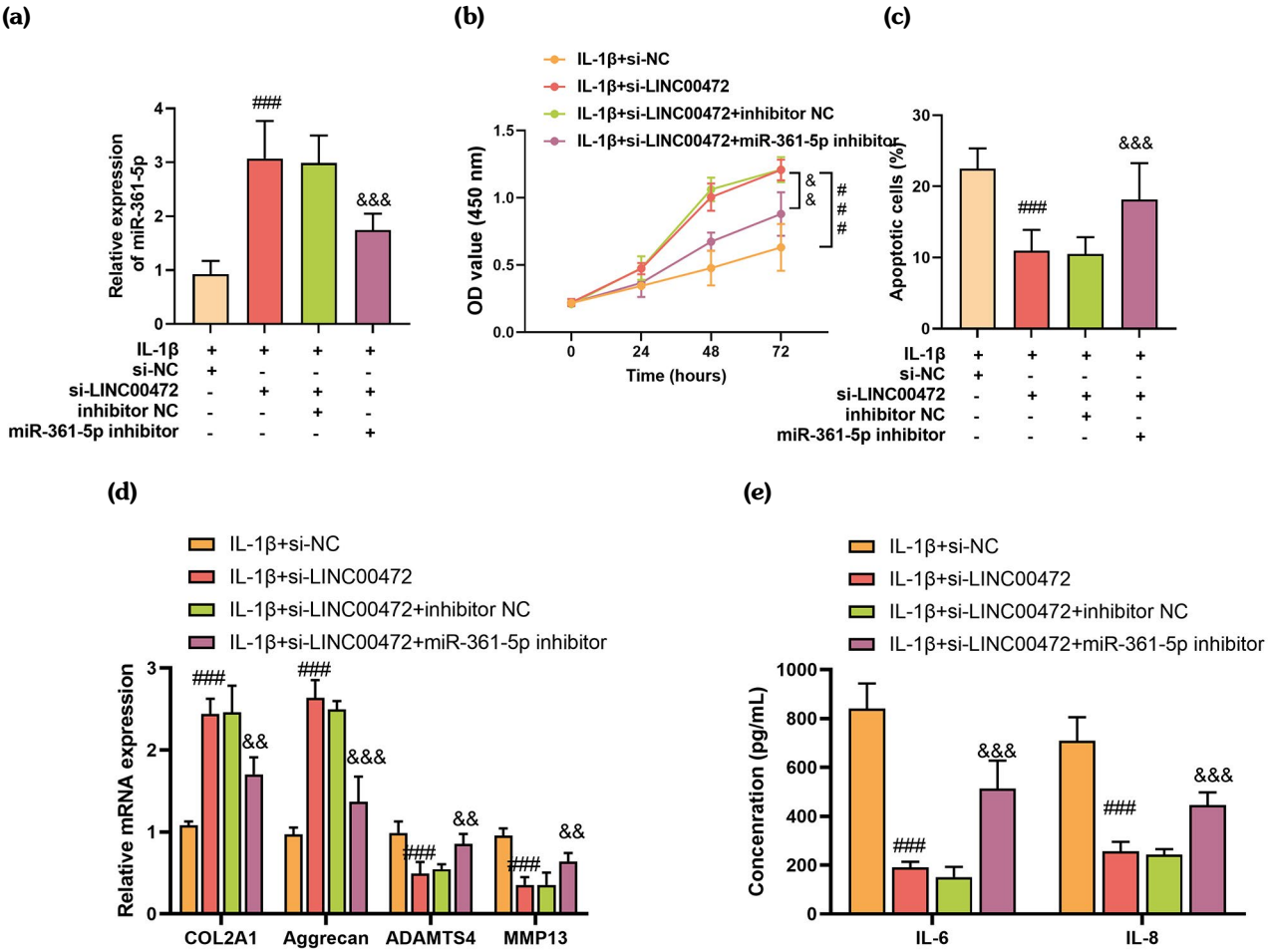

Figure 5. LINC00472 and miR-361-5p coregulated chondrocyte damage. **(a-e)** After transfection with si-NC, si-LINC00472, NC inhibitor, and miR-361-5P inhibitor, chondrocytes were treated with 10 ng/mL IL-1β. **(a)** miR-361-5P levels were determined by RT-qPCR. **(b)** Differences in cell viability between different groups. CCK-8 assay was used to assess cell viability. **(c)** Differences in apoptosis between groups. Flow cytometry was used to detect apoptosis. **(d)** The level of factors associated with ECM degradation was determined by RT-qPCR. E. IL-6 and IL-8 levels were determined by ELISA.

IL: Interleukin; RT-qPCR: Real-time quantitative reverse transcription polymerase chain reaction; ELISA: Enzyme-linked immunosorbent assay; ### p<0.001 *vs.* IL-1β+si-NC; && p<0.01 *vs.* IL-1β+si- LINC00472; &&& p<0.001 *vs.* IL-1β+si- LINC00472.

MECP2-WT was increased (Figure 6d). Both miR-361-5p and MECP2 could be enriched in Ago2 (Figure 6e). Compared to healthy subjects, MECP2 levels in OA patients were highly expressed (Figure 6f), and MECP2 levels were negatively or positively correlated with miR-361-5p or LINC00472 levels (Figures 6g, h). In addition, IL-1β significantly affected the level of MECP2, and with the increase of IL-1β concentration and the passage of treatment time, the level of MECP2 showed a dose-dependent increase (Figures 6i, j). Finally, we found that after the downregulation of LINC00472, MECP2 levels decreased; however, this result was reversed by the addition of miR-361-5p inhibitor (Figure 6k).

DISCUSSION

Osteoarthritis is the most common joint disease characterized by damage to the articular cartilage and involves the entire joint tissue, culminating in the degeneration of the articular cartilage, fibrosis, and fracture.[19] Cartilage tissue

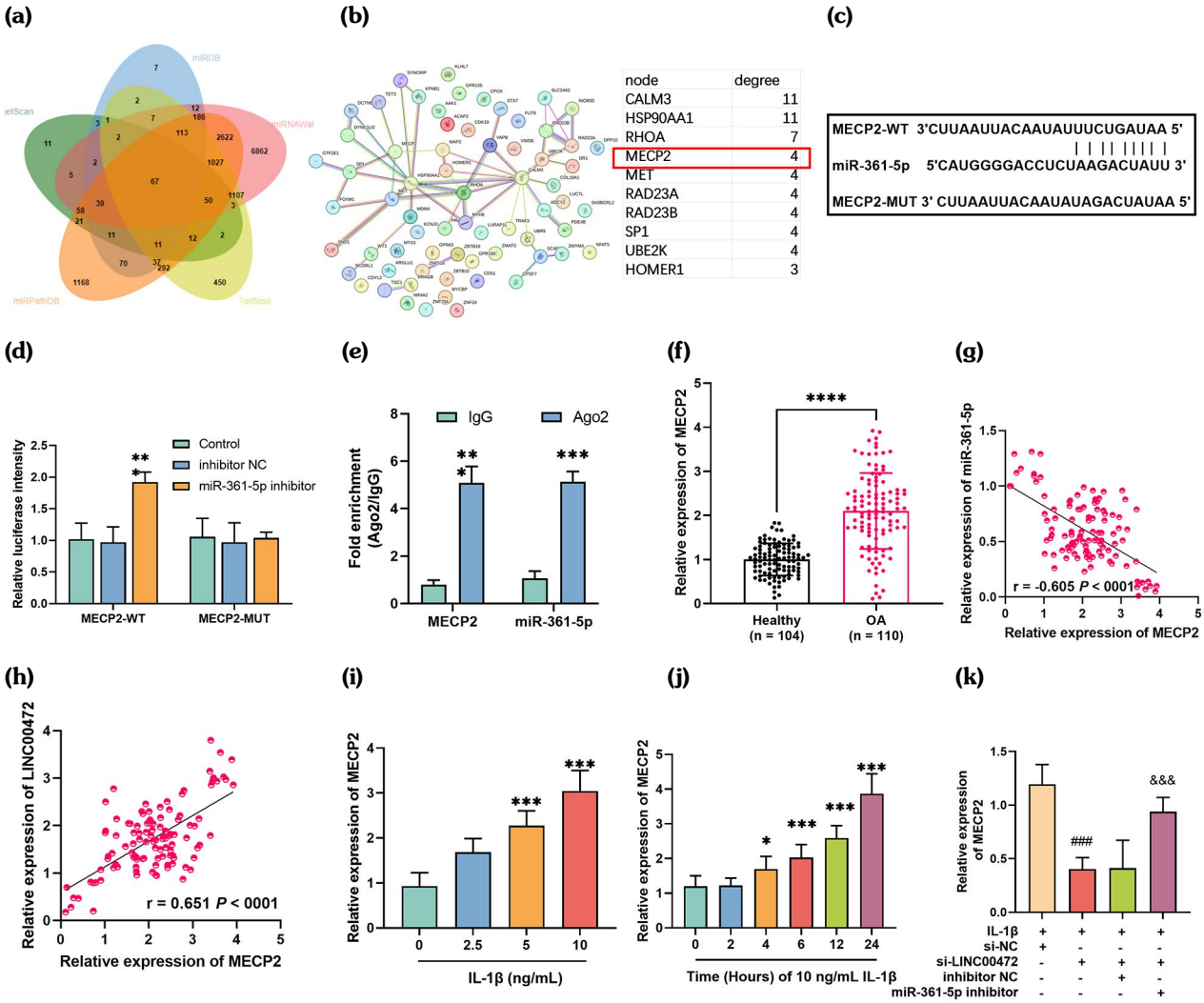

Figure 6. miR-361-5p targeted MECP2. **(a)** The overlapping target genes for miR-361-5p. **(b)** The PPI network of miR-361-5p relative genes in the STRING database. **(c)** Complementary binding site of miR-361-5p to MECP2 and mutation site of miR-361-5p. **(d-e)** Dual luciferase analysis experiments and RIP analysis verified the relationship between miR-361-5p and MECP2. F. MECP2 levels were measured by RT-qPCR. **(g, h)** The correlation between MECP2 with miR-361-5p expression and MECP2 with LINC00472 expression was analyzed by Pearson correlation analysis. **(i, j)** Expression levels of MECP2 at different IL-1β concentrations and different treatment times. **(k)** After transfection with si-NC, si-LINC00472, NC inhibitor, and miR-361-5P inhibitor, chondrocytes were treated with 10 ng/mL IL-1β. MECP2 levels were determined by RT-qPCR.

IgG: Immunoglobulin G; OA: Osteoarthritis; IL: Interleukin; RT-qPCR: Real-time quantitative reverse transcription polymerase chain reaction; RIP: RNA immunoprecipitation; RNA: Ribonucleic acid; * p<0.05; *** p<0.001; **** p<0.0001 *vs.* Control; ### p<0.001 *vs.* IL-1β. &&& p<0.001 *vs.* IL-1β+si-LINC00472.

consists mainly of chondrocytes with ECM.[20] The balance between anabolism and catabolism is necessary to maintain chondrocyte homeostasis, and loss of chondrocyte homeostasis leads to OA.[21] LncRNAs were found to be involved in catabolic processes in OA, such as cellular autophagy, apoptosis, cellular inflammation, and ECM degradation.[22,23] According to previous studies, LINC00472 is involved in various nonmalignant diseases such as atherosclerosis, primary biliary cholangitis, atrial fibrillation, acute liver injury, and cardiac dysfunction, in addition to its role as an oncostatic factor in a variety of cancers.[24] However, whether

LINC00472 is involved in the development of OA remains to be confirmed.

In our study, we found that the expression of LINC00472 was increased in OA patients compared to the healthy group, LINC00472 levels were positively correlated with disease severity, and the receiver operating characteristic curves indicated that LINC00472 could be utilized to differentiate OA patients and healthy individuals. Therefore, LINC00472 may be a diagnostic biomarker for OA. LINC00472 has proinflammatory effects in various inflammatory models, such as the lipopolysaccharide (LPS)-induced septic liver injury model[17] and the neuropathic pain model,[18] where LINC00472 was shown to promote the expression of inflammatory factors. Thus, the progression of OA may be associated with increased expression of LINC00472 in OA patients. To further explore the action of LINC00472 involved in the regulation of OA, we constructed a cellular model of OA by treating human chondrocytes with IL-1β. In colorectal cancer and lung adenocarcinoma, LINC00472 was shown to promote apoptosis.[25,26] In addition, LINC00472 knockdown significantly attenuated the LPS-induced decrease in cardiomyocyte viability and reversed LPS-mediated apoptosis and inflammatory responses in cardiomyocytes.[27] In our study, we also found that the downregulation of LINC00472 inhibited the decrease in cell viability, apoptosis, cellular inflammation, and ECM degradation induced by IL-1β. Based on the role of LINC00472 in chondrocyte apoptosis, ECM degradation, and inflammatory responses, we hypothesized that LINC00472 may be a novel target for OA diagnosis and treatment.

Recent studies have shown that lncRNA interacts with miRNAs and jointly participates in many diseases.[28] In this study, LINC00472 sponged miR-361-5p. miR-361-5p has been shown to be aberrantly expressed in patients with rheumatoid arthritis and chronic gouty arthritis, promote fibroblast-like synoviocyte proliferation, and inhibit apoptosis.[29,30] In OA-related studies, miR-361-5p was decreased in OA chondrocytes and synovial tissues, which was able to suppress inflammations in chondrocytes by inhibiting the secretion of inflammatory factors.[31] Our findings are consistent with the literature. In our study, miR-361-5p was decreased in OA patients, and miR-361-5p inhibitor suppressed cell viability and promoted apoptosis, ECM degradation, and inflammatory responses through negative regulation of si-LINC00472. Our findings demonstrate that miR-361-5p inhibits the development of OA, whereas LINC00472 exacerbates OA by sponging miR-361-5p.

MECP2 is located at Xq28 and is a multifunctional gene with ubiquitous expression.[32] We found that miR-361-5p targeted MECP2. Both miR-361-5p and MECP2 could be enriched in Ago2, and miR-361-5p inhibitor enhanced the luciferase activity of MECP2. MECP2 plays a proinflammatory role in a variety of diseases such as neuroinflammation,[33] acute liver injury,[34] rheumatoid arthritis,[35] and OA.[36] In OA, upregulation of MECP2 triggered chondrocyte injury,[37] whereas downregulation of MECP2 attenuated chondrocyte apoptosis, ECM degradation, and inflammatory responses.[36] Therefore, we hypothesized that MECP2 could promote the progression of OA. Consistently, we found that MECP2 levels were elevated in OA patients, and IL-1β induced upregulation of MECP2. Correlation analysis showed that MECP2 levels were positively correlated with LINC00472 levels and negatively correlated with miR-361-5p levels. MECP2 had a role in promoting chondrocyte apoptosis, ECM degradation and proinflammatory effects in OA. We assumed that LINC00472 upregulated MECP2 expression via sponge adsorption of miR-361-5p, and high levels of MECP2 promoted chondrocyte apoptosis, ECM degradation, and inflammation in OA patients, which in turn exacerbated the progression of OA.

This study had some limitations. The in vitro design constituted a limitation, and future studies should explore the relationship between LINC00472 and OA progression in animal models of OA. In addition, the LINC00472/miR-361-5p/MECP2 axis should be explored in the clinical diagnosis and treatment of OA.

In conclusion, LINC00472 plays an important role in OA progression, and LINC00472 promotes chondrocyte injury and aggravates OA development through the miR-361-5p/MECP2 axis. This study may provide a new research target for the diagnosis and treatment of OA.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conceptualization, data curation, formal analysis, investigation, methodology, Z.L., W.Z.; Resources, software, validation, visualization, writing-original draft, Z.L.; Project administration, supervision, visualization, writing-review & editing: W.Z.

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