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Circ_0045714 alleviates TNF-α-induced chondrocyte injury and extracellular matrix degradation through miR-218-5p/HRAS axis

Haitao Jiang[1] · Jian Dai[1] · Cheng Zhang[1] · Hailang Sun[1] · Xiaoming Tang[1]

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Abstract

Emerging evidence suggests that dysregulated circular RNAs (circRNAs) play a pivotal role in osteoarthritis (OA). Circ_0045714 is a functional circRNAs, and has been revealed to involve in the process of OA. However, the molecular mechanisms by which circ_0045714 regulates OA progression are not thoroughly elucidated. Circ_0045714 expression was decreased in OA and TNF-α-induced chondrocytes, ectopic overexpression of circ_0045714 abolished TNF-α-induced cell apoptosis, inflammation, extracellular matrix (ECM) degradation promotion and proliferation inhibition. In a mechanical study, circ_0045714 targeted miR-218-5p, and miR-218-5p overexpression reversed the effects of circ_0045714 on TNF-α-induced chondrocytes. Besides that, HRAS was a target of miR-218-5p, and HRAS knockdown attenuated the protective effects of miR-218-5p inhibition on TNF-α-induced chondrocyte dysfunction. Additionally, circ_0045714 could regulate HRAS expression via miR-218-5p in chondrocytes. Up-regulation of circ_0045714 suppressed TNF-α-induced chondrocyte growth inhibition, inflammation, and ECM degradation via miR-218-5p/HRAS axis, suggesting a novel insight into the pathogenesis of OA and the potential protective role of circ_0045714 in the occurrence and development of OA.

Keywords circ_0045714 · miR-218-5p · HRAS · Chondrocyte · Osteoarthritis

Introduction

Osteoarthritis (OA) is a common age-related degenerative joint disease characterized by a hallmark symptom of pain, even results in physical disability (Glyn-Jones et al. 2015). The main features of OA are the loss of articular cartilage and the formation of new bone on joint surface (Wang et al. 2011). Increased degradation of the extracellular matrix (ECM) in the cartilage is a key factor of articular cartilage destruction (Okada and Okada 2009; Rahmati et al. 2017). Chondrocytes are the sole cell type in the articular system, and play a vital role in maintaining cartilage structure, function and ECM production (Bi et al. 2019). Besides that, it has been reported that low-grade and chronic inflammation also contribute to the pathophysiology of OA (Daghestani and Kraus 2015; Malfait 2016). Thus, studies on chondrocyte phenotype switching, inflammation, and ECM degradation are essential for the development of novel and effective treatment for OA.

Circular RNAs (circRNAs) are a novel class of RNAs with special circular structures, which render then resist RNA degradation pathways (Memczak et al. 2013; Zhou et al. 2018). CircRNAs show high stability and specie-, tissue-, cell-specific expression profiles in eukaryotic cells (Fischer and Leung 2017). Increasing findings have indicated that circRNAs have significant roles in complex human pathologies through involving in regulating biological processes, such as apoptosis, proliferation, metabolism, inflammation and vascularization (Marques-Rocha et al. 2015; Yu and Kuo 2019). Notably, circRNAs are strongly linked to the genesis and progression of OA (Li et al. 2018). For example, Zhou et al. showed circRNA.33,186 markedly promoted the proliferation and ECM degradation of interleukin (IL)-1β-treated chondrocytes *in vitro* and destabilized medial meniscus (DMM)-induced OA process in mice *in vivo* (Zhou et al. 2019). Shen et al. revealed that circSERPINE2 up-regulation could alleviate chondrocyte apoptosis and catabolism of ECM *in vitro* and hinder OA progression in rabbit models *in vivo* (Shen et al. 2019). Circ_0045714 is a novel functional circRNAs, a recent study has suggested that circ_0045714 exerted a protective role in OA process through regulating

✉ Xiaoming Tang
ttkwsk@163.com

[1] Department of Orthopedics, The Affiliated No. 1 People's Hospital of Nangjing Medical University, No.1, Huanghe West Road, Huaiyin District, Huai'an 223300, China

chondrocyte phenotype changes and ECM synthesis (Li et al. 2017). However, large-scale investigations of circ_0045714 in OA initiation and development were not yet reported.

Increasing findings have reported that tumor necrosis factor-α (TNF-α) plays an important role in promoting catabolic and destructive processes of human chondrocytes in joints tissues, which accelerates the occurrence and development of OA (Bi et al. 2019; Daghestani and Kraus 2015). Hence, TNF-α-induced chondrocytes were used to elucidate the role and molecular mechanism of circ_0045714 in cell growth, inflammation and ECM degradation in OA cell models *in vitro*, which might provide a novel insight into the pathogenesis of OA.

Materials and methods

Clinical tissues

OA cartilage tissues were isolated from 17 OA patients underwent total knee replacement surgery. The diagnosis of OA patients were based on guideline of the American College of Rheumatology criteria. Control articular cartilage tissues were obtained from 9 patients without OA or rheumatoid arthritis history by the traumatic emergency amputation. All cartilage tissues were immediately frozen in the liquid nitrogen and stored at -80 . This study was approved by the Ethics Committee of The Affiliated No. 1 People's Hospital of Nangjing Medical University and every enrolled individual and their families was informed of the study and signed informed consent.

Cell culture

Human articular chondrocytes were isolated from normal articular cartilage samples. The normal cartilage tissues subtracted with excess fibrous connective tissue were cut into about 1–2 mm^3 size, and subjected to sequential digestion with both 0.2% trypsin and 0.2% collagenase type II at 37 for 30 min and 6 h, respectively, after washing with PBS (Sigma-Aldrich, Saint Louis, MO, USA) containing penicillin sodium and gentamicin. Afterwards, cell suspension was filtered, centrifuged and washed several times with PBS. Finally, the isolated articular chondrocytes were collected and grown in the Dulbecco's modified Eagle medium (DMEM, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Gibco, Rockville, MD, USA) at 37 in a humidified atmosphere 5% CO_2. Chondrocytes passaged 2–3 times was used for subsequent analyses. Besides that, HEK293T cells were obtained from Beijing Institute for Cancer Research Collection (Beijing, China) and grown in the same medium and condition.

The chondrocytes were treated with different concentrations of TNF-α (0, 5, 10 and 20 ng/mL, Sigma-Aldrich) for 12 h when reaching 80% confluences. Untreated chondrocytes were used as control.

Quantitative real-time polymerase chain reaction (qRT-PCR)

TRIzol® Reagent (Life Technologies, Carlsbad, CA, USA) was employed to extract total RNA from frozen patient samples and cells. After that, the RNA was reversely-transcribed by the Reverse Transcription System Kit (Takara, Kusatsu, Japan) to generate the complementary DNA (cDNA), then subjected to qRT-PCR using the SYBR green PCR kit (Takara). The relative fold changes were evaluated using the $2^{-\Delta\Delta Ct}$ method and normalized to U6 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers for qRT-PCR were listed: circ_0045714: F 5'-CCGAGGTGCCCATTCCTG-3', R 5'-TTGGTGCAGTTGCCTTTCG-3'; miR-218-5p: F 5'-TTGCGGATGGTTCCGTCAAGCA-3', R 5'-ATCCAGTGCAGGGTCCGAGG-3'; GADPH: F 5'-CCCACATGGCCTCCAAGGAGTA-3', R 5'-GTGTACATGGCAACTGTGAGGAGG-3'; HRas Proto-Oncogene GTPase (HRAS): F 5'-TGCTTCAGTTTGAACTACCCTG-3', R 5'-GCCCAGTGCTGATAGCCAG-3'; U6: F 5'-AAAGCAAATCATCGGACGACC-3', R 5'-GTACAACACATTGTTTCCTCGGA-3'.

Cell counting kit-8 (CCK-8) assay

After indicated treatment and/or transfection, chondrocytes were seeded into each well of 96-well plates (2×10^4 cells/well), then cell proliferation was evaluated by detecting the absorbance at 450 nm after the addition of 10 μL CCK-8 solution (5 mg/mL, Sigma-Aldrich) per well for 2 h.

Flow cytometry

Chondrocytes treated with TNF-α for 12 h were maintained in 96-well plates, followed by transfection with assigned vectors for 48 h. Thereafter, chondrocytes were resuspended in binding buffer to make the density at 1×10^6/mL after washing with PBS, followed by double-staining with 10 μL annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) (BD Biosciences, Franklin Lakes, NJ, USA) away from light. Finally, cell apoptosis was analyzed uisng the BD FACSCalibur flow cytometry (BD Biosciences).

Cell transfection

The pcDNA3.1 vector encoding circ_0045714 and empty vector (Vector), specific siRNA targeting HRAS (si-HRAS) and negative control siRNA (si-NC), and the mimic and

inhibitor of miR-218-5p (miR-218-5p or anti-miR-218-5p) and their negative controls (miR-NC or anti-miR-NC) were procured from GenePharma Company (Shanghai, China). Then 10 nM vectors, or 45 nM siRNAs, or 50 mM miRNAs mimics or inhibitors were transfected into chondrocytes using Lipofectamine 2000 reagent (Invitrogen).

Western blot

Total proteins were extracted with RIPA lysis buffer from tissues and chondrocytes, 50–100 μg of protein extracts were loaded onto 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis for separating and then transferred to the polyvinylidene difluoride membranes (Roche Life Sciences, Indianapolis, IN). The membranes were blocked with 5% skimmed milk, and incubated with the appropriate primary antibody at 4 overnight and HRP-conjugated secondary antibody for 1 h. The antigen-antibody complex on the membrane were visualized by sensitive electrochemiluminescence (ECL) method. The antibodies used in this study: anti-Type collagen II (1:1000, ab188570), anti-Aggrecan (1:1000, ab36861), matrix metallopeptidase 13 (MMP-13) (1:5000, ab39012), anti-thrombospondin motif-4 (ADAMTS-4) (1:1000, ab185722), interleukin (IL)-6 (IL-6) (1:1000, ab6672), IL-8 (1:1000, ab18672), anti-HRAS (1:1000, ab97488) and anti-GAPDH (1:1000, ab8245), all purchased from Abcam (Cambridge, MA, USA).

Dual-luciferase reporter assay

The wild type and mutant sequences of circ_0045714 or HRAS 3'-UTR with the binding sites for miR-218-5p were cloned into the pmirGLO vector (Promega, Madison, WI, USA) to establish wild-type pmirGLO vectors (circ_0045714-WT, HRAS 3'-UTR-WT) or mutant pmirGLO vectors (circ_0045714-MUT, HRAS 3'-UTR-MUT). Then these constructed luciferase report vectors together with miR-218-5p mimic or miR-NC mimic were co-transfected into chondrocytes using Lipofectamine 2000 (Invitrogen). At last, the Dual Luciferase Reporter Assay Kit (Promega) was employed to determine the relative luciferase activities.

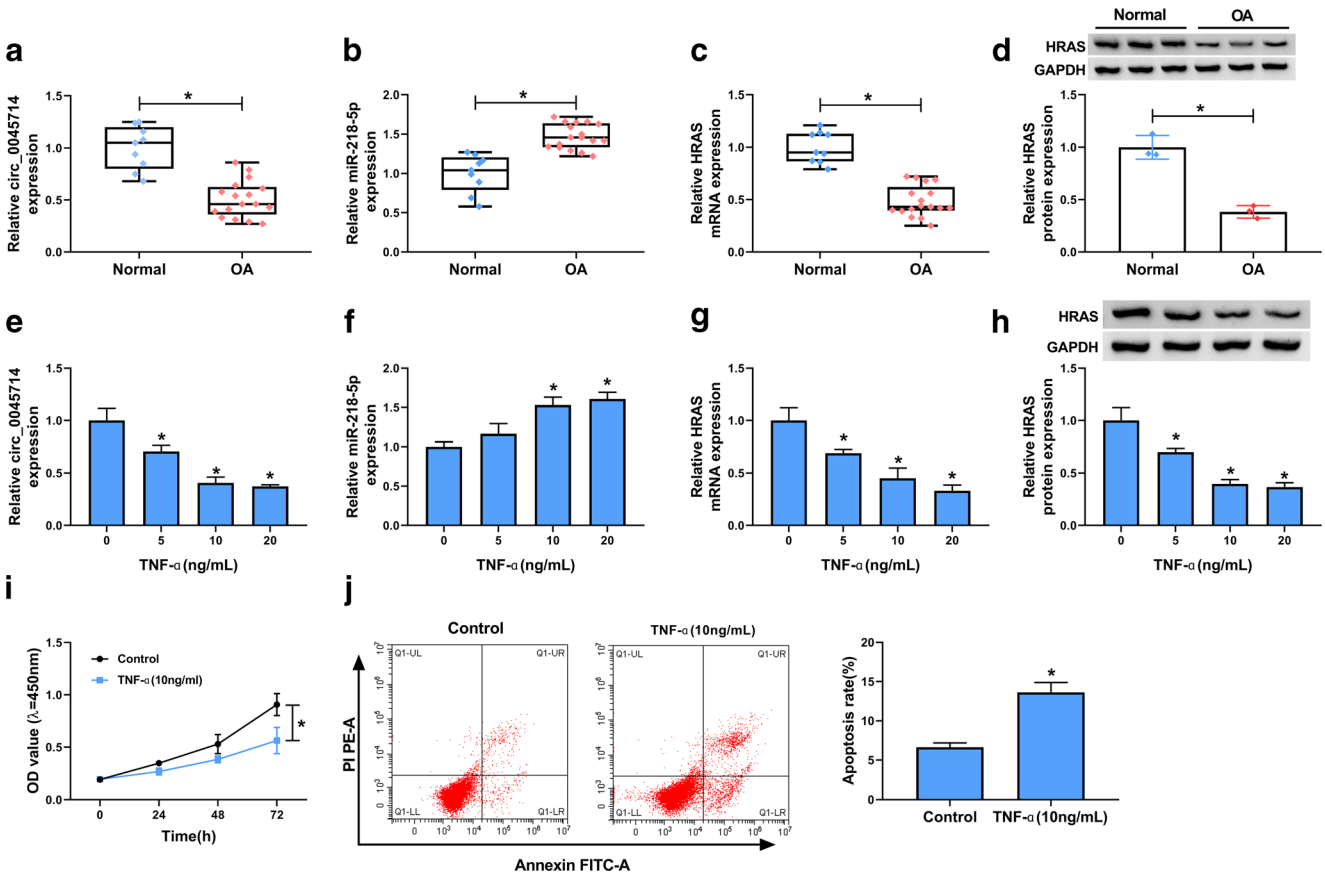
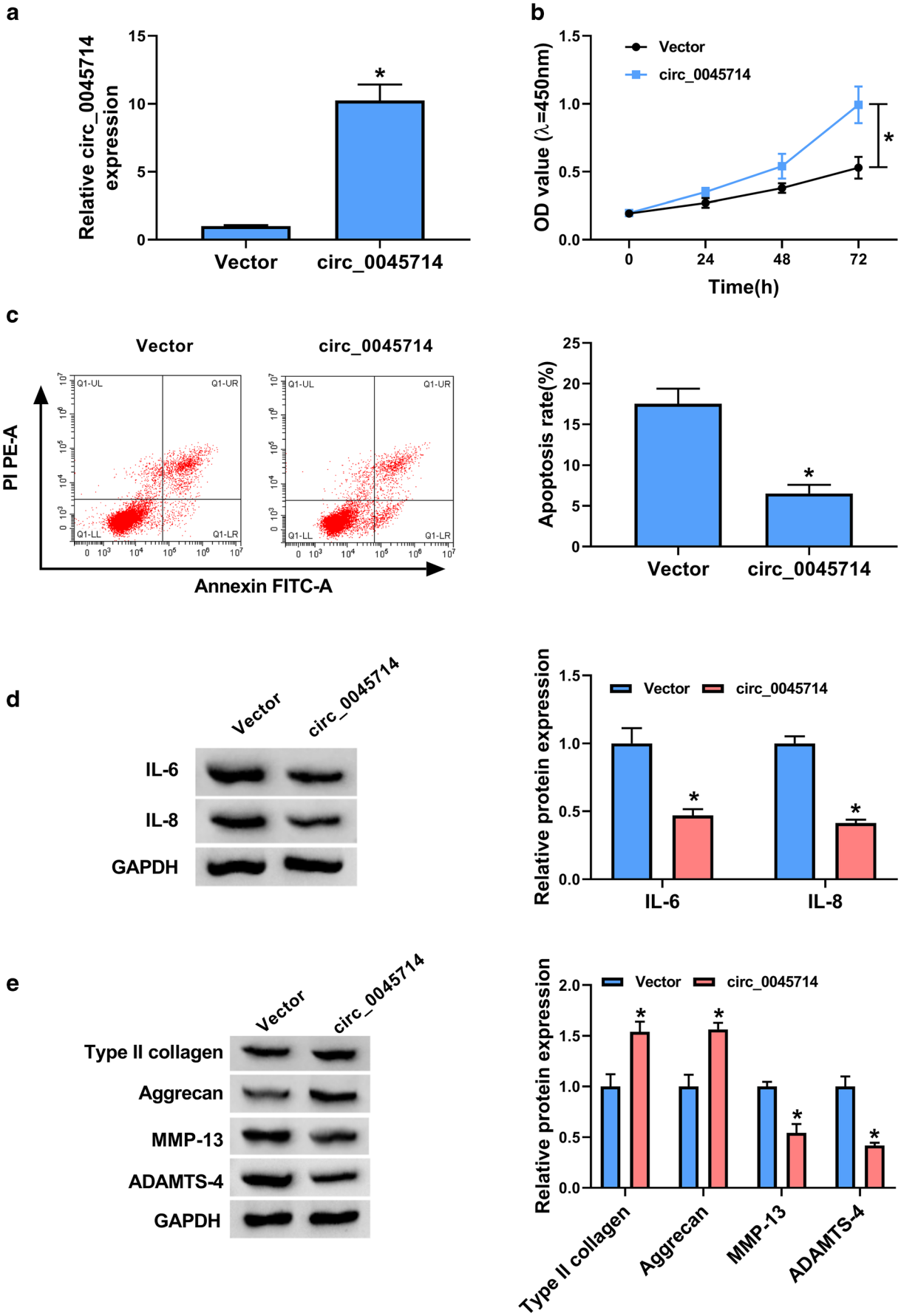


Fig. 1 The expression profiles of circ_0045714, miR-218-5p, and HRAS in OA and TNF-α-induced chondrocytes. **a-d** qRT-PCR or western blot analysis of the expression of circ_0045714, miR-218-5p, and HRAS in cartilage tissues from OA patients or normal controls. (E-H) qRT-PCR or western blot analysis of circ_0045714, miR-218-5p, and HRAS expression of in chondrocytes treated with different concentrations of TNF-α (0, 5, 10 and 20 ng/mL) for 12 h. (I) CCK-8 assay of the proliferation of chondrocytes treated with 10 ng/mL TNF-α for 12 h. **j** Flow cytometry analysis of the apoptosis rate of TNF-α-induced chondrocytes. *$P < 0.05$


a
Relative circ_0045714 expression
15
10
5
0
*
Vector
circ_0045714
b
OD value (λ=450nm)
1.5
1.0
0.5
0.0
Vector
circ_0045714
*
0
24
48
72
Time(h)
c
Vector
circ_0045714
PI PE-A
Annexin FITC-A
Q1-UL
Q1-UR
Q1-LL
Q1-LR
Apoptosis rate(%)
25
20
15
10
5
0
*
Vector
circ_0045714
d
Vector
circ_0045714
IL-6
IL-8
GAPDH
Relative protein expression
1.5
1.0
0.5
0.0
Vector
circ_0045714
*
IL-6
IL-8
e
Vector
circ_0045714
Type II collagen
Aggrecan
MMP-13
ADAMTS-4
GAPDH
Relative protein expression
2.0
1.5
1.0
0.5
0.0
Vector
circ_0045714
*
Type II collagen
Aggrecan
MMP-13
ADAMTS-4

◀ **Fig. 2** Circ_0045714 overexpression attenuates TNF-α-induced chondrocyte dysfunction. **a** qRT-PCR of circ_0045714 expression in chondrocytes transfected with circ_0045714 or Vector. Chondrocytes were treated with 10 ng/mL TNF-α for 12 h, followed by transfection with circ_0045714 or Vector. **b** CCK-8 assay of cell proliferation. **c** Flow cytometry analysis of cell apoptosis. **d, e** Western blot analysis of the levels of IL-6, IL-8, MMP-13, ADAMTS-4, Type II collagen and Aggrecan in cells. *$P < 0.05$

Statistical analysis

Data from at least three independent experiments were taken and presented as mean ± standard deviation (SD). Statistical analyses were conducted under GraphPad Prism 7 software. Comparisons between different groups were performed using Student's *t* test and one-way analysis of variance analysis (ANOVA) as appropriate. The correlation between two variables was evaluated using the Spearman's correlation analysis. $P < 0.05$ were considered as statistically significant.

Results

The expression profiles of circ_0045714, miR-218-5p, HRAS in OA and TNF-α-induced chondrocytes

To reveal the potential role of circ_0045714, miR-218-5p, HRAS in chondrocytes, we firstly detected their expression in OA and TNF-α-induced chondrocytes. As shown in Fig. 1a-d, we found circ_0045714 and HRAS expression levels was lower, while miR-218-5p level was higher in OA cartilage tissues than that in normal cartilage tissues. In the dose-dependent experiment, we treated chondrocytes with different concentrations of TNF-α (0, 5, 10 and 20 ng/mL) and observed that compared with the untreated cells, TNF-α treatment caused the significant decrease of circ_0045714 and HRAS levels and increase of miR-218-5p level in normal chondrocytes (Fig. 1e-h), suggesting that circ_0045714, miR-218-5p, and HRAS might be inducible responsive factors to inflammatory stimuli in chondrocytes. In the following studies, chondrocytes were treated with 10 ng/mL TNF-α for 12 h to establish OA cell model *in vitro*, the CCK-8 assay and flow cytometry analysis suggested TNF-α suppressed cell proliferation and induced cell apoptosis (Fig. 1i, j), indicating the successful establishment of AS cell models *in vitro*.

Circ_0045714 overexpression attenuates TNF-α-induced chondrocyte dysfunction

Next, we investigated the impact of circ_0045714 on the growth, inflammation and extracellular matrix (ECM) degradation of chondrocytes. Circ_0045714 overexpression vector was designed to increase circ_0045714 expression in chondrocytes, as expected, a significant elevation of circ_0045714 expression was observed in cells after transfection (Fig. 2a). Then, we discovered that circ_0045714

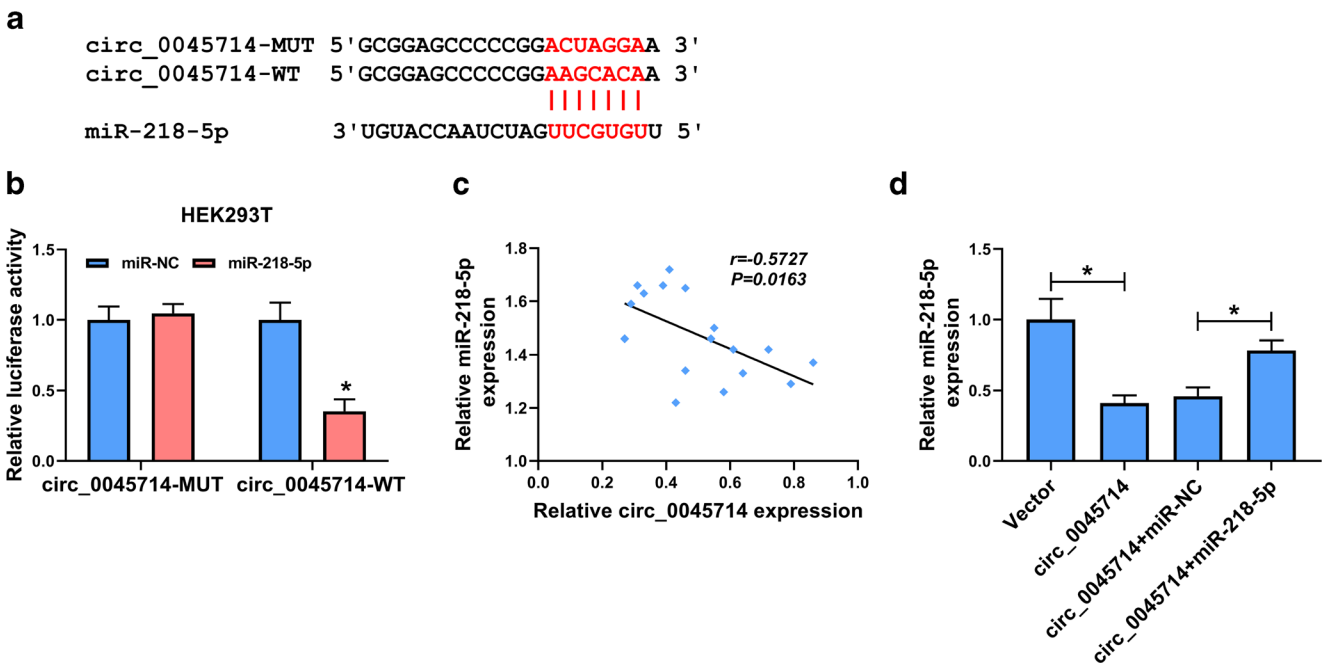
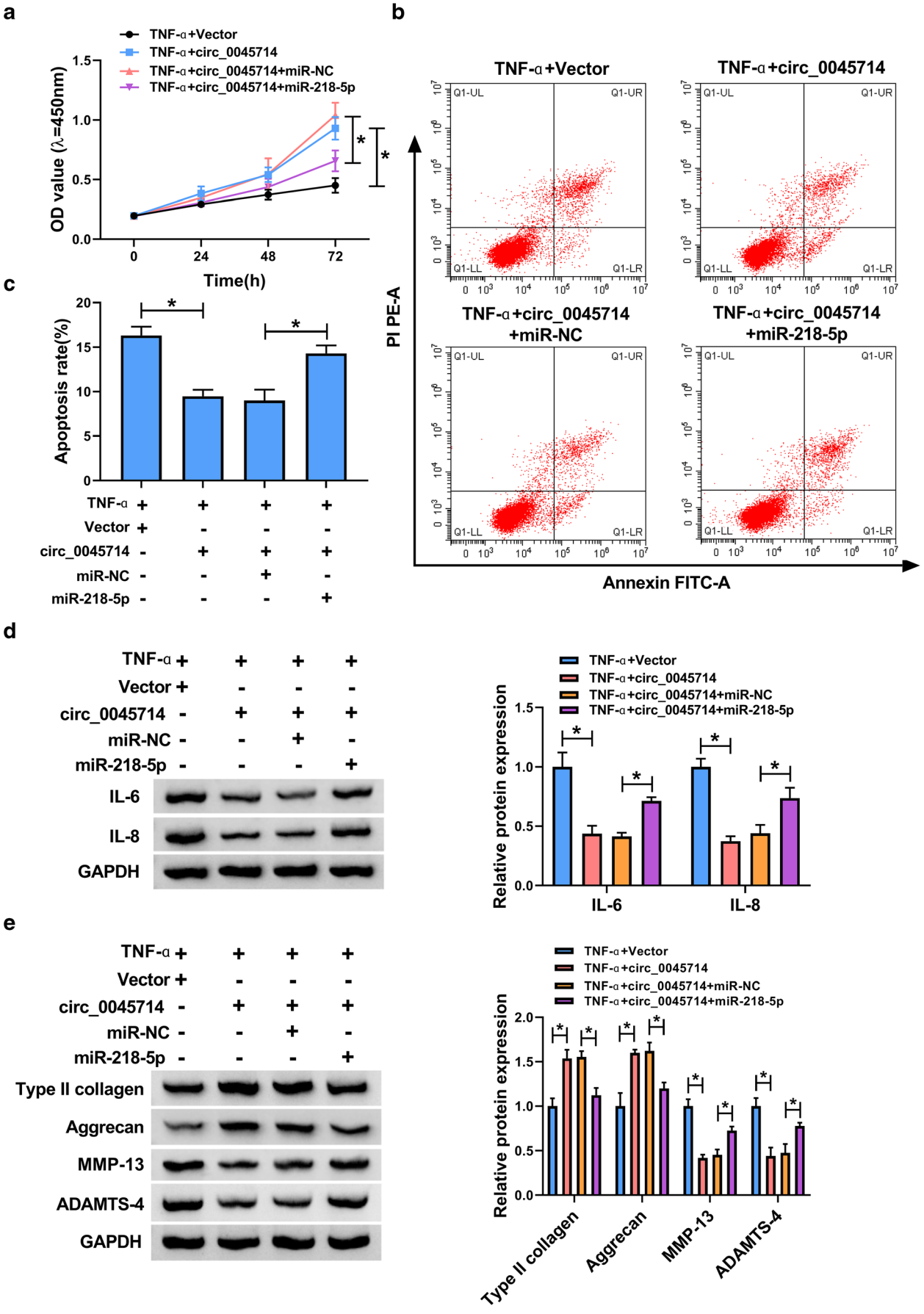


Fig. 3 MiR-218-5p is a target of circ_0045714. **a** Potential sites targeted by miR-218-5p in circ_0045714 sequence. **b** Luciferase activity analysis in HEK293T cells co-transfected with the reporter vectors and the indicated miRNAs using the dual-luciferase reporter assay. **c** Correlation analysis between miR-218-5p and circ_0045714 expression in cartilage tissues using Spearman's correlation analysis. **d** qRT-PCR of miR-218-5p expression in chondrocytes transfected with Vector, circ_0045714, circ_0045714 + miR-NC, circ_0045714 + miR-218-5p. *$P < 0.05$


a
TNF-α+Vector
TNF-α+circ_0045714
TNF-α+circ_0045714+miR-NC
TNF-α+circ_0045714+miR-218-5p
OD value (λ=450nm)
Time(h)
b
TNF-α+Vector
TNF-α+circ_0045714
TNF-α+circ_0045714 +miR-NC
TNF-α+circ_0045714 +miR-218-5p
PI PE-A
Annexin FITC-A
Q1-UL
Q1-UR
Q1-LL
Q1-LR
c
Apoptosis rate(%)
TNF-α
Vector
circ_0045714
miR-NC
miR-218-5p
d
IL-6
IL-8
GAPDH
Relative protein expression
e
Type II collagen
Aggrecan
MMP-13
ADAMTS-4
GAPDH

◀ **Fig. 4** Circ_0045714 attenuates TNF-α-induced chondrocyte dysfunction by binding to miR-218-5p. Chondrocytes were treated with 10 ng/mL TNF-α for 12 h, followed by transfection with Vector, circ_0045714, circ_0045714 + miR-NC, circ_0045714 + miR-218-5p. **a** Cell proliferation analysis using CCK-8 assay. **b, c** Cell apoptosis analysis using flow cytometry. **d, e** Levels detection of IL-6, IL-8, MMP-13, ADAMTS-4, Type II collagen and Aggrecan in cells using western blot analysis. $*P < 0.05$

overexpression in TNF-α-induced chondrocytes reversed TNF-α-evoked cell proliferation arrest (Fig. 2b) and apoptosis promotion (Fig. 2c). Meanwhile, western blot assay showed TNF-α induced the release of IL-6 and IL-8 in chondrocytes, which was attenuated by circ_0045714 up-regulation (Fig. 2d); besides that, it was also exhibited ectopic overexpression of circ_0045714 increased the levels of Type II collagen and Aggrecan, while reduced the levels of MMP-13 and ADAMTS-4 in TNF-α-induced chondrocytes (Fig. 2e). Taken together, circ_0045714 overexpression abolished TNF-α-triggered chondrocyte growth inhibition, inflammation activation and ECM degradation, thus affecting the process of OA.

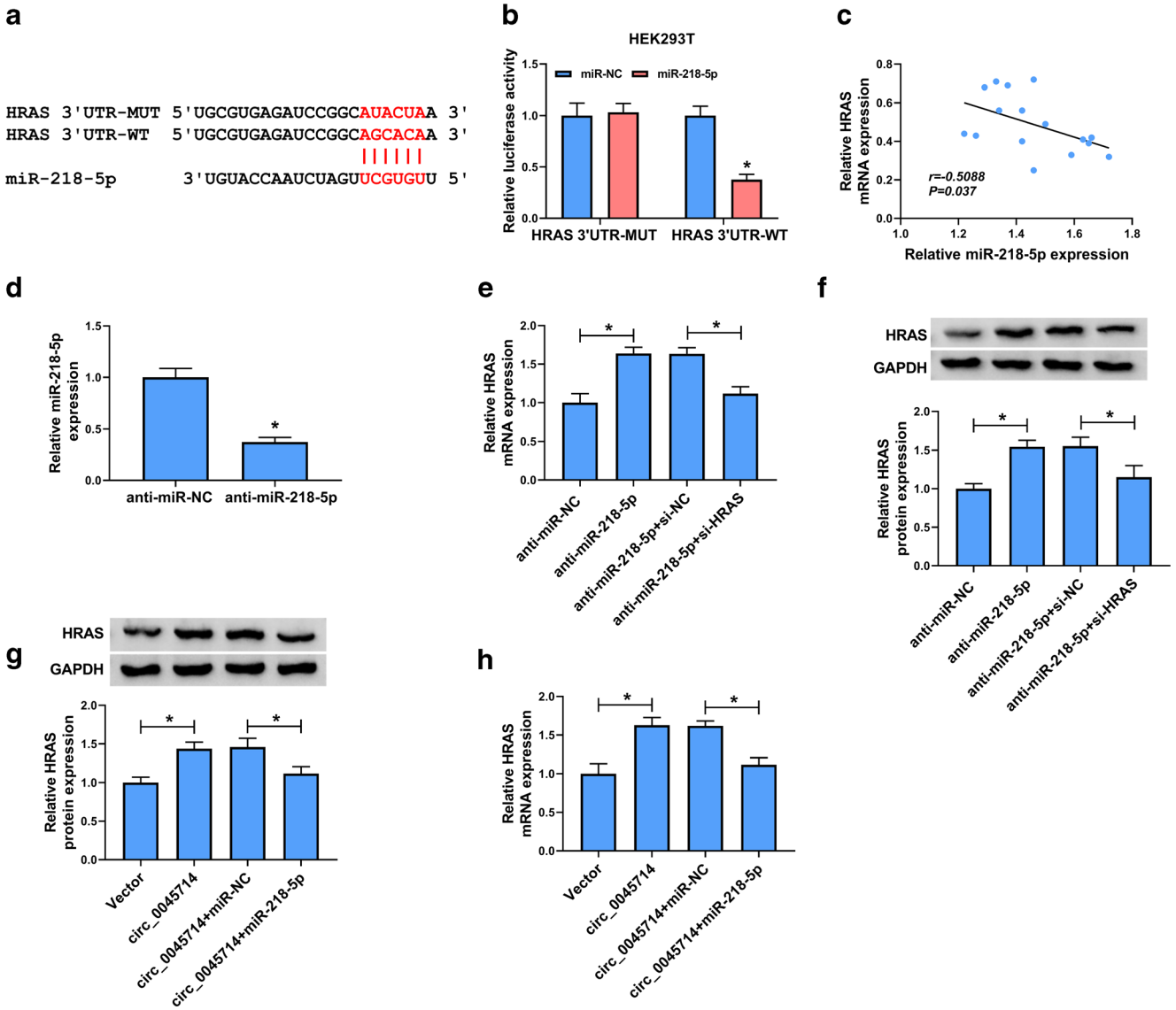
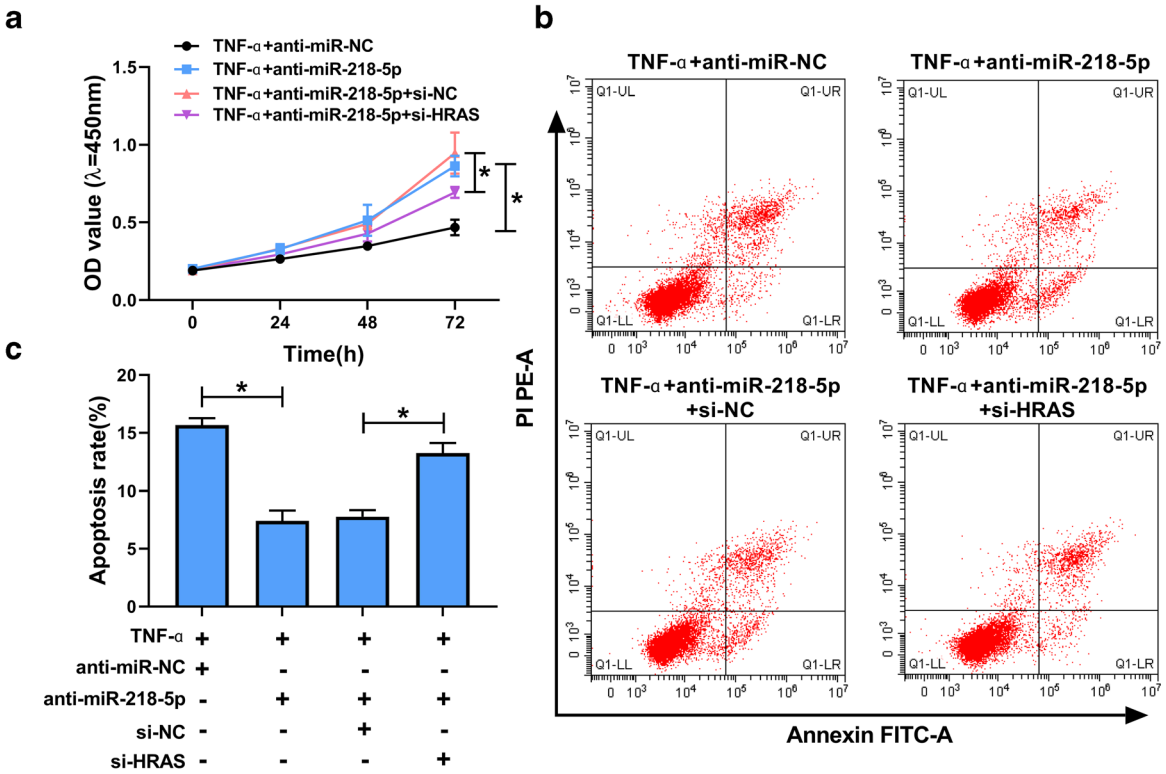


Fig. 5 Circ_0045714 can indirectly regulate HRAS through targeting miR-218-5p. **a** Potential binding sites between miR-218-5p and HRAS. **b** Luciferase activity analysis in HEK293T cells co-transfected with the reporter vectors and the indicated miRNAs using the dual-luciferase reporter assay. **c** Correlation analysis between miR-218-5p and HRAS expression in cartilage tissues using Spearman's correlation analysis. **d** qRT-PCR of miR-218-5p expression in chondrocytes transfected with anti-miR-NC or anti-miR-218-5p. **e, f** qRT-PCR or western blot analysis of HRAS expression in chondrocytes transfected with anti-miR-NC, anti-miR-218-5p, anti-miR-218-5p + si-NC, or anti-miR-218-5p + si-HRAS. **g, h** qRT-PCR or western blot analysis of HRAS expression in chondrocytes transfected with Vector, circ_0045714, circ_0045714 + miR-NC, or circ_0045714 + miR-218-5p. $*P < 0.05$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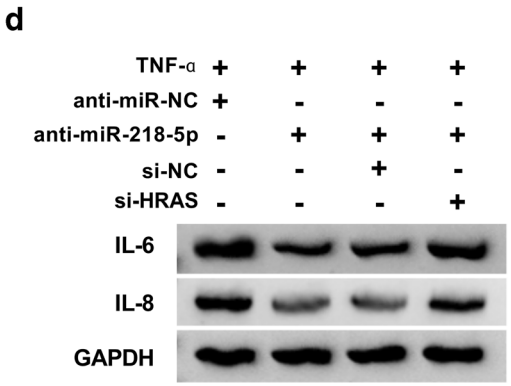
TNF-α+anti-miR-NC
TNF-α+anti-miR-218-5p
TNF-α+anti-miR-218-5p+si-NC
TNF-α+anti-miR-218-5p+si-HRAS
OD value (λ=450nm)
Time(h)
b
TNF-α+anti-miR-NC
TNF-α+anti-miR-218-5p
TNF-α+anti-miR-218-5p +si-NC
TNF-α+anti-miR-218-5p +si-HRAS
PI PE-A
Annexin FITC-A
Q1-UL
Q1-UR
Q1-LL
Q1-LR
c
Apoptosis rate(%)
TNF-α
anti-miR-NC
anti-miR-218-5p
si-NC
si-HRAS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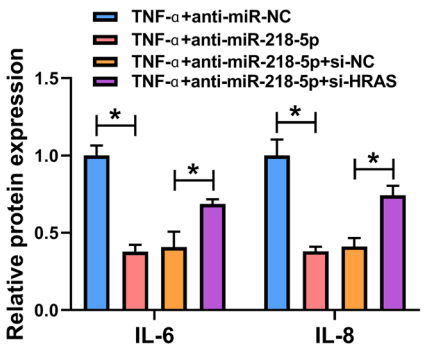
TNF-α+anti-miR-NC
TNF-α+anti-miR-218-5p
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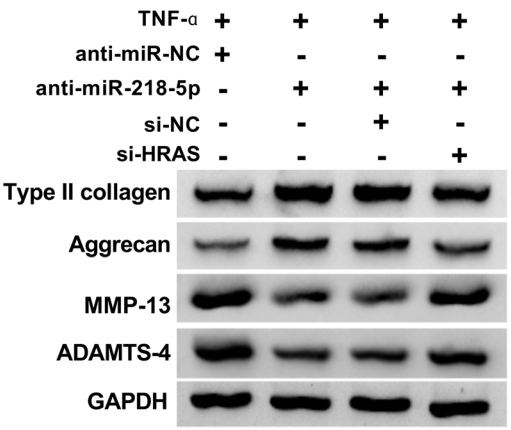
TNF-α+anti-miR-218-5p+si-HRAS
Relative protein expression
IL-6
IL-8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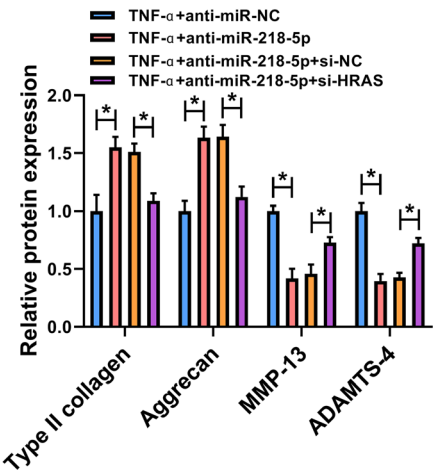
si-HRAS
Type II collagen
Aggrecan
MMP-13
ADAMTS-4
GAPDH


TNF-α+anti-miR-NC
TNF-α+anti-miR-218-5p
TNF-α+anti-miR-218-5p+si-NC
TNF-α+anti-miR-218-5p+si-HRAS
Relative protein expression
Type II collagen
Aggrecan
MMP-13
ADAMTS-4

◀ **Fig. 6** MiR-218-5p suppression abrogates TNF-α-induced chondrocyte dysfunction by targeting HRAS. Chondrocytes were treated with 10 ng/mL TNF-α for 12 h, followed by transfection with anti-miR-NC, anti-miR-218-5p, anti-miR-218-5p + si-NC, or anti-miR-218-5p + si-HRAS. **a** CCK-8 assay of cell proliferation. **b**, **c** Flow cytometry analysis of cell apoptosis. **d**, **e** Western blot analysis of the levels of IL-6, IL-8, MMP-13, ADAMTS-4, Type II collagen and Aggrecan in cells. $^{*}P < 0.05$

MiR-218-5p is a target of circ_0045714

To further exploration of the potential mechanism of circ_0045714 in OA progression, the potential target miRNAs of circ_0045714 were firstly searched using the Starbase on lines database, and miR-218-5p was selected to be the possible target of circ_0045714 (Fig. 3a). Then the dual-luciferase reporter assay confirmed the direct interaction between miR-218-5p and circ_0045714, evidenced by the reduction of the luciferase activity in HEK293T cells co-transfected with circ_0045714-WT and miR-218-5p (Fig. 3b). MiR-218-5p expression was found to be negatively correlated with circ_0045714 in OA cartilage tissues (Fig. 3c); parallelly, miR-218-5p expression was also decreased by the overexpression of circ_0045714 in chondrocytes, which was rescued by the introduction of miR-218-5p mimic (Fig. 3d). Altogether, circ_0045714 directly targeted miR-218-5p and negatively modulated its expression.

Circ_0045714 attenuates TNF-α-induced chondrocyte dysfunction by binding to miR-218-5p

Given the axis of circ_0045714/miR-218-5p, we then studied whether circ_0045714 achieved its function via miR-218-5p. The CCK-8 and flow cytometry assays displayed circ_0045714 up-regulation promoted chondrocyte growth under TNF-α stress, however, this effect was abolished by the increase of miR-218-5p (Fig. 4a-c). The expression levels of IL-6 and IL-8 were suppressed in circ_0045714-increased chondrocytes treated with TNF-α, which was dramatically blocked when miR-218-5p was overexpressed (Fig. 4d). Additionally, increasing miR-218-5p also reversed circ_0045714-evoked inhibition of ECM degradation in TNF-α-treated chondrocytes, reflected by the decrease of Type II collagen and Aggrecan levels and increase of MMP-13 and ADAMTS-4 levels (Fig. 4e). These findings demonstrated that MALAT1 might suppress TNF-α-triggered chondrocyte growth inhibition, inflammation activation and ECM degradation via miR-218-5p.

Circ_0045714 can indirectly regulate HRAS through targeting miR-218-5p

The potential target mRNAs of miR-218-5p were also searched using bioinformatics analysis (TargetScan), and HRAS was found to have the complementary binding sites on miR-218-5p (Fig. 5a). Dual-luciferase reporter assay exhibited miR-218-5p overexpression markedly reduced the luciferase activity of HRAS 3'UTR-WT in HEK293T cells (Fig. 5b), proving that miR-218-5p targeted HRAS. HRAS expression was observed to be negatively correlated with miR-218-5p (Fig. 5c); additionally, when we reduced the expression of miR-218-5p in chondrocytes by introducing anti-miR-218-5p into cells (Fig. 5d), it was found miR-218-5p inhibition elevated the level of HRAS, both at mRNA and protein levels, in chondrocytes, while this elevation was reverted by following si-HRAS transfection (Fig. 5e, f). Thus, miR-218-5p negatively regulated HRAS expression. What's more, we also observed circ_0045714 could increase the expression of HRAS in chondrocytes, which was abolished by miR-218-5p overexpression (Fig. 5g, h), suggesting circ_0045714 could indirectly regulate HRAS via miR-218-5p in chondrocytes.

MiR-218-5p suppression abrogates TNF-α-induced chondrocyte dysfunction by targeting HRAS

To probe whether miR-218-5p/HRAS axis was associated with TNF-α-induced chondrocyte dysfunction, anti-miR-218-5p or (and) si-HRAS were transfected into chondrocytes after TNF-α treatment. We found miR-218-5p inhibition promoted cell proliferation and suppressed cell apoptosis in TNF-α-induced chondrocytes, which were reversed by HRAS knockdown (Fig. 6a-c). Western blot analysis miR-218-5p inhibition attenuated TNF-α-induced elevation of IL-6, IL-8, MMP-13 and ADAMTS-4 levels and down-regulation of Type II collagen and Aggrecan levels in chondrocytes, while HRAS silencing dramatically abated these effects (Fig. 6d, e). Collectively, inhibition of miR-218-5p alleviated TNF-α-triggered chondrocyte growth inhibition, inflammation activation and ECM degradation through targeting HRAS.

Discussion

OA is the most common type of joint disease and is a leading cause of impaired mobility, physical disability, pain, and stiffness among the elderly population (Hu et al. 2018). Currently, the main medical care is based on moderating pain symptoms, however, no effective drug can stop the progression of this disease to date; joint replacement is still the primary treatment for end-stage OA patients, whereas the limited lifespan of prostheses, poor functional outcomes and high socioeconomic cost limits the clinical application and efficacy of this technique (Blaney Davidson et al. 2017; Litwic et al. 2013).

Therefore, it is imperative to better understand pathogenesis of OA to explore effective therapeutic strategies for OA.

At present, circRNAs have been shown to have critical roles in both chondrocyte development and cartilage homeostasis, circRNAs regulation in the joint has been shown to reduce OA in animal models, suggesting the potential therapeutic roles of circRNAs in OA (Li et al. 2018; Wu et al. 2019; Zhou et al. 2019). With OA onset, chondrocytes undergo multiple changes, in terms of proliferation and apoptosis. In this study, circ_0045714 was found to be decreased in OA cartilage tissues and TNF-α-induced chondrocytes, when up-regulating the level of circ_0045714 in TNF-α-induced chondrocytes, we found circ_0045714 overexpression reduced cell apoptosis and promoted cell proliferation. Besides, its overexpression also reduced the release of inflammatory chemokine IL-6 and IL-8, thus suppressing cell inflammation. The dynamic equilibrium between anabolism and catabolism in the ECM is the main cause of tissue destruction and remodeling process in OA-affected articular cartilage (Sandell and Aigner 2001). MMP-13 and ADAMTS-4 were identified as major cartilage-degrading enzymes, and type collagen II and aggrecan were known as major collagen components in the cartilage matrix (Zhang et al. 2019). This study discovered that ectopic overexpression of circ_0045714 abolished TNF-α-induced accumulation of anabolic factors MMP-13 and ADAMTS-4 and reduction of Type collagen II and Aggrecan catabolic factors in chondrocytes, thereby impeding ECM catabolism. Taken together, circ_0045714 could reduce TNF-α-evoked cell growth inhibition, inflammation, and ECM degradation in chondrocytes, suggesting that circ_0045714 might have protective effects on OA process.

It has been documented that circRNAs can involve in human diseases via 'miRNA sponge', circRNAs function as miRNA sponges by acting as competing endogenous RNA (ceRNA) to regulate their downstream functions (Du et al. 2017; Hansen et al. 2013). Hence, the target miRNAs of circ_0045714 were investigated. This study confirmed that circ_0045714 directly bound to miR-218-5p. One previous study uncovered that miR-218-5p was highly expressed in OA, especially in severe OA; besides that, its down-regulation triggered matrix synthesis and proliferation in OA chondrocytes *in vitro* and reduced cartilage degradation in mice model *in vivo* (Lu et al. 2017). In this work, we also observed an increase of miR-218-5p level in TNF-α-induced chondrocytes, and inhibition of miR-218-5p reversed TNF-α-induced death, inflammation, and ECM degradation in chondrocytes. More importantly, it was proved that miR-218-5p abrogated the protective effects of circ_0045714 on TNF-α-induced chondrocyte dysfunction. Thus, a potential circ_0045714/miR-218-5p axis in TNF-α-induced OA process was identified.

HRAS is one of the RAS isoforms, the mutation or up-regulation of which has been reported in several cancers, and is considered as an oncogene and known for its proliferative role in cancers (Sugita et al. 2018). HRAS is an important effectors in a number of signaling cascades, which can modulate the activation of mitogen activated protein kinase (MAPK) signaling and the PI3K pathway, thus affecting cell differentiation, survival and proliferation (Aksamitiene et al. 2012; Xing 2010). Recently, it was uncovered that HRAS was lowly expressed in OA tissues (Fu et al. 2015; Lü et al. 2020), and restoration of HRAS in IL-1β-induced OA chondrocytes reduced cell apoptosis, senescence and ECM degradation (Lü et al. 2020). In the present work, we proved that HRAS knockdown attenuated the rescue effects of miR-218-5p inhibition on TNF-α-induced chondrocyte apoptosis, inflammation and imbalance of ECM degradation. Besides, we also demonstrated that circ_0045714 could indirectly up-regulate HRAS expression level, which was reversed by miR-218-5p overexpression.

In conclusion, these findings demonstrated that circ_0045714 protected chondrocytes from TNF-α-induced cell apoptosis, inflammation and ECM degradation through miR-218-5p/HRAS axis, indicating a novel insight into the pathology of OA and a potential therapeutic targets for OA treatment.

Compliance with ethical standards

Disclosure of interest The authors declare that they have no conflicts of interest.

References

Aksamitiene E, Kiyatkin A, Kholodenko BN (2012) Cross-talk between mitogenic Ras/MAPK and survival PI3K/Akt pathways: a fine balance. Biochem Soc Trans 40(1):139–146

Bi J, Cai W, Ma T, Deng A, Ma P, Han Y et al (2019) Protective effect of vildagliptin on TNF-α-induced chondrocyte senescence. IUBMB Life 71(7):978–985

Blaney Davidson EN, van Caam AP, van der Kraan PM (2017) Osteoarthritis year in review 2016: biology. Osteoarthr Cart 25(2): 175–180

Daghestani HN, Kraus VB (2015) Inflammatory biomarkers in osteoarthritis. Osteoarthr Cart 23(11):1890–1896

Du WW, Zhang C, Yang W, Yong T, Awan FM, Yang BB (2017) Identifying and Characterizing circRNA-Protein Interaction. Theranostics 7(17):4183–4191

Fischer JW, Leung AK (2017) CircRNAs: a regulator of cellular stress. Crit Rev Biochem Mol Biol 52(2):220–233

Fu M, Huang G, Zhang Z, Liu J, Zhang Z, Huang Z et al (2015) Expression profile of long noncoding RNAs in cartilage from knee osteoarthritis patients. Osteoarthr Cart 23(3):423–432

Glyn-Jones S, Palmer AJ, Agricola R, Price AJ, Vincent TL, Weinans H et al (2015) Osteoarthr Lancet 386(9991):376–387

Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK et al (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495(7441):384–388

Hu J, Wang Z, Shan Y, Pan Y, Ma J, Jia L (2018) Long non-coding RNA HOTAIR promotes osteoarthritis progression via miR-17-5p/FUT2/β-catenin axis. Cell Death Dis 9(7):711

Li BF, Zhang Y, Xiao J, Wang F, Li M, Guo XZ et al (2017) Hsa_circ_0045714 regulates chondrocyte proliferation, apoptosis and extracellular matrix synthesis by promoting the expression of miR-193b target gene IGF1R. Hum Cell 30(4):311–318

Li HZ, Lin Z, Xu XH, Lin N, Lu HD (2018) The potential roles of circRNAs in osteoarthritis: a coming journey to find a treasure. Biosci Rep 38(5):BSR20180542

Litwic A, Edwards MH, Dennison EM, Cooper C (2013) Epidemiology and burden of osteoarthritis. Br Med Bull 105:185–199

Lu J, Ji ML, Zhang XJ, Shi PL, Wu H, Wang C et al (2017) MicroRNA-218-5p as a potential target for the treatment of human osteoarthritis. Mol Ther 25(12):2676–2688

Lü G, Li L, Wang B, Kuang L (2020) LINC00623/miR-101/HRAS axis modulates IL-1β-mediated ECM degradation, apoptosis and senescence of osteoarthritis chondrocytes. Aging 12(4):3218–3237

Malfait AM (2016) Osteoarthritis year in review 2015: biology. Osteoarthr Cart 24(1):21–26

Marques-Rocha JL, Samblas M, Milagro FI, Bressan J, Martínez JA, Marti A (2015) Noncoding RNAs, cytokines, and inflammation-related diseases. FASEB J 29(9):3595–3611

Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A et al (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495(7441):333–338

Okada A, Okada Y (2009) [Progress of research in osteoarthritis. Metalloproteinases in osteoarthritis]. Clin Calcium 19(11):1593–1601

Rahmati M, Nalesso G, Mobasheri A, Mozafari M (2017) Aging and osteoarthritis: Central role of the extracellular matrix. Ageing Res Rev 40:20–30

Sandell LJ, Aigner T (2001) Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res 3(2):107–113

Shen S, Wu Y, Chen J, Xie Z, Huang K, Wang G et al (2019) CircSERPINE2 protects against osteoarthritis by targeting miR-1271 and ETS-related gene. Ann Rheum Dis 78(6):826–836

Sugita S, Enokida H, Yoshino H, Miyamoto K, Yonemori M, Sakaguchi T et al (2018) HRAS as a potential therapeutic target of salirasib RAS inhibitor in bladder cancer. Int J Oncol 53(2):725–736

Wang Q, Rozelle AL, Lepus CM, Scanzello CR, Song JJ, Larsen DM et al (2011) Identification of a central role for complement in osteoarthritis. Nat Med 17(12):1674–1679

Wu Y, Lu X, Shen B, Zeng Y (2019) The therapeutic potential and role of miRNA, lncRNA, and circRNA in osteoarthritis. Curr Gene Ther 19(4):255–263

Xing M (2010) Genetic alterations in the phosphatidylinositol-3 kinase/Akt pathway in thyroid cancer. Thyroid 20(7):697–706

Yu CY, Kuo HC (2019) The emerging roles and functions of circular RNAs and their generation. J Biomed Sci 26(1):29

Zhang Y, Wang F, Chen G, He R, Yang L (2019) LncRNA MALAT1 promotes osteoarthritis by modulating miR-150-5p/AKT3 axis. Cell Biosci 9:54

Zhou R, Wu Y, Wang W, Su W, Liu Y, Wang Y et al (2018) Circular RNAs (circRNAs) in cancer. Cancer Lett 425:134–142

Zhou ZB, Huang GX, Fu Q, Han B, Lu JJ, Chen AM et al (2019) circRNA.33186 contributes to the pathogenesis of osteoarthritis by sponging miR-127-5p. Mol Ther 27(3):531–541