AGT, targeted by miR-149-5p, promotes IL-6-induced inflammatory responses of chondrocytes in osteoarthritis via activating the JAK2/STAT3 pathway

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Abstract

Objective

Angiotensinogen (AGT) and miR-149-5p were differentially expressed genes in the osteoarthritis (OA), but their functional contribution to this disease is unclear. Our study aimed to illustrate their relevance to OA pathology and chondrocytic inflammation responses.

Methods

In this study, a total of 32 healthy donors and 56 OA patients were recruited for cartilage tissues, and interleukin (IL)-6-stimulated human chondrocyte-articular (HC-a) cells were used as an in vitro OA model.

Results

RT-qPCR and western blot assays demonstrated that AGT was upregulated in OA cartilage tissues while miR-149-5p was downregulated. Using a loss-of-function assay and inhibitor treatment, we found that AGT knockdown inhibited the increase of IL-1β, matrix metalloproteinase (MMP)-13 and nitrite in IL-6-induced chondrocytes through blocking the renin-angiotensin system (RAS). The prediction (TargetScan) and validation (mutant and luciferase reporter assays) of the interaction between AGT and miR-149-5p indicated that miR-149-5p directly regulated inflammatory responses in OA chondrocytes by binding to AGT. Furthermore, using overexpression and inhibitor treatment experiments, our study proved that JAK2/STAT3 was activated in OA tissues, and AGT regulated OA inflammation via activating the JAK2/STAT3 pathway.

Conclusion

Our study demonstrated that AGT, modulated and directly bond by miR-149-5p, promoted the IL-6-induced inflammatory responses in OA via JAK2/STAT3 pathway.

Key words

angiotensinogen, miR-149-5p, renin-angiotensin system, osteoarthritis, JAK2/STAT3 pathway

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Introduction

Osteoarthritis (OA), a kind of degenerative joint disease, is widely prevalent in older populations and is a leading cause of chronic disability (1, 2). A recent investigation showed approximately 250 million people worldwide suffer this burdensome disorder (3). Crucially, OA leads to notable health problems and prominent socioeconomic costs (4, 5). Therefore, it is worth studying the pathogenesis of OA and related therapy managements.

Current studies have characterised OA as the degeneration and loss of cartilage, secondary hyperosteogeny, changes in subchondral bone, synovitis and other joint tissues (6, 7). Cartilage is one of the most susceptible tissue structures in OA due to its avascularity; thus, cartilage destruction is considered to be the hallmark of OA (8). Previous studies indicated that the ectopic inflammatory response of chondrocytes is a crucial factor that originates and amplifies the disease (9). Abnormal levels of various factors, including nitric oxide (NO), free radicals, matrix metalloproteinases (MMPs) and cytokines, are involved in OA pathology (10). Chondrocytic inflammation induces and exacerbates the release of OA-related inflammatory mediators, MMP-13, interleukin (IL)-1β, prostaglandin E2 (PGE2) and NO included (11, 12).

Previous studies indicated that the renin-angiotensin system (RAS) is a regulatory hormonal cascade that represents a master regulator of blood pressure and fluid homeostasis (13, 14). Yamagishi and colleagues recently reported the association between RAS and hypertrophic chondrocyte differentiation, and also confirmed that the promotive activation of RAS induced osteoarthritic responses in the knee joint (15). Scientists determined that RAS members, such as angiotensin-converting enzyme (ACE), and angiotensin II (ANG II) receptors, are expressed in bone tissue (16). Besides, the clinical evidence related to rheumatoid arthritis (RA) and OA further indicates ectopic ACE and renin expression in synovial stroma, as well as the activation of angiotensin II type 1 receptor (AT1R) and AT2R in chondrocytes under pathological condition (17, 18). These investigations showed that RAS played a facilitative role in the initiation and development of OA. However, little is known about the potential mechanism of RAS in modulating the osteoarthritic inflammation response in chondrocytes.

Angiotensinogen (AGT) is a member of RAS, and its encoded protein acts as the substrate for renin. ANG I, the protein product cleaved by renin, is then cleaved by ACE to eventually form ANG II (19, 20). The importance of AGT in cardiovascular diseases was validated in numerous associated studies. However, the role of AGT in OA pathological process has rarely been mentioned. A recent microarray-based analysis discovered that AGT was differentially expressed and participated in protein interaction network in both early and late stages of knee OA (21). Thus, we reasonably inferred that AGT should be a crucial target in OA development.

MicroRNA (miRNA) is a single-stranded non-coding RNA, approximately 22 nucleotides long (22). MiRNA post-transcriptionally and negatively regulates the expression of target genes, and emerges as a hotspot in myriad research. Also, miRNA plays a crucial role in the regulation of various physiological and pathological processes, OA included (23, 24). A recent study that used integrated bioinformatics analysis on OA cartilage samples revealed that miR-149-5p was differentially expressed (25). However, the effect of abnormal miR-149-5p expression on OA development remains unclear.

In the present study, to better understand the molecular mechanism by which AGT regulates the OA inflammatory response and the role of miR-149-5p in the pathological process, we detected the expressions of AGT and miR-149-5p in cartilage tissues and cells under OA conditions. Besides, we conducted loss-of-function and overexpression experiments, and analysed the relevant regulatory mechanisms. The evidence might be conducive to provide a novel exploring target for further OA diagnosis and therapy.

Materials and methods

Patient samples

In this study, we collected 56 OA cartilages from OA patients who underwent

total hip joint re-placement surgery at the first hospital of Lanzhou university All the patients were diagnosed and evaluated by three certified rheumatologists (K/L Grade I-II, 29 cases; K/L Grade III-IV, 27 cases), with no complications suffered, and had not acceped any kind of targeted drug treatment. Thirty-two matched normal cartilages were obtained during the surgery for traumatic fractures, and these patients (K/L Grade 0) have no history of joint disease and their cartilages were macroscopically normal. All subjects provided written informed consent. The Ethic Committee of Clinical Investigation of the First Hospital of Lanzhou University approved our research. Besides, all the experiments that involved human specimens were performed in accordance with the Declaration of Helsinki. The cartilage tissue was immediately frozen using liquid nitrogen after surgery and cryopreserved at - 80°C.

Cell culture and treatment

The chondrocyte cell line, HC-a, was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, MA, USA) that contained 10% Fetal Bovine Serum (FBS) (Invitrogen, MA, USA) with a humidified atmosphere at 37°C with 5% CO_2.

The HC-a cells stimulation with IL-6 (ACRO Biosystems, MA, USA) was conducted after cell transfection or inhibitor treatment (JAK/STAT inhibitor, WP1066 MedChemExpress, NJ, USA). Also, we performed time and concentration gradient experiments to determine the optimal IL-6 stimulation concentration and treatment time.

Reverse transcription quantitative PCR (RT-qPCR)

TRIzol reagent (Invitrogen, MA, USA) was used to extract total RNA following the manufacturer's protocol. After quantifying with 1.5% agarose gel electrophoresis, RNA quality was evaluated using a NanoDrop 8000 (Thermo Fisher, MA, USA). For RT-qPCR analysis, Omniscript reverse transcription kit (Qiagen, Dusseldorf, Germany) was utilised to synthesise cDNA from 1 μg purified RNA that was pre-treated with DNase I (Invitrogen, MA, USA). RT-qPCR was performed on an ABI7500 quantitative PCR instrument (Applied Biosystems, Foster City, USA) using DyNamo SYBR1 Green qPCR kit (Takara, Dalian, China) according to the manufacturer's instructions. The reaction was performed as followed: 1 min at 95°C, 35 cycles of 20 s at 95°C, 10 s at 56°C and 15 s at 72°C, and finally held at 4°C. Using GAPDH as the normalisation gene, the relative level of mRNA was calculated by $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

The levels of protein expressions (AGT, ANG II, AT1R, p-JAK2, JAK2, p-STAT3, STAT3) were assessed by western blot assay. Briefly, total proteins of cartilage tissues and HC-a cells were extracted using cell lysis buffer (Cell Signaling, Danvers, USA). Protein was collected and quantified using BCA protein assay kit (Beyotime, Shanghai, China). Equal amounts of protein were separated with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then, proteins were transferred onto 5% skim milk blocked polyvinylidene difluoide (PVDF) membranes (Millipore, Massachusetts, USA) and incubated with primary antibodies 4°C overnight. GAPDH was the internal control group. After washed with TBS buffer, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell Signaling, Danvers, USA) for another 2 h at room temperature. Finally, the bands were visualised using ECL method and quantified by a Chemi Doc XRS Imaging System, Bio-Rad (California, USA).

Cell transfection

Small interfering RNAs (siRNAs), mimics and inhibitors were provided by Invitrogen (Carlsbad, CA). PcDNA3.1 used for overexpression experiments was purchased from Generay Biotech (Shanghai, China). In line with the manufacturer's protocol, the transfections on chondrocytes were performed using Lipofectamine 2000 reagent (Invitrogen, MA, USA). Negative control (NC) siRNA was used for control.

Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (R&D Systems, Minneapolis, USA) were used to detect IL-1β and MMP-13 contents in the concentrated cell culture supernatants from every group, according to the manufacturer's instruction.

Determination of NO

The nitrite content measured by Griess reagent (Sigma-Aldrich, Darmstadt, Germany) according to the manufacturer's protocol was utilised to estimate NO levels in different treatment groups.

Prediction of miRNA binding and mutation assay

In order to predict the binding between miR-149-5p and AGT, we submitted the gene symbol or gene ID of both on the online tool, TargetScan Human release 7.2 (http://www.targetscan.org/vert_72/).

For the mutation on 3`UTR of AGT, we synthesised specific primers based on the miR-149-5p binding motif in AGT and introduced mutation sequences into primers. Next, the wild type and mutant 3`UTR of AGT were cloned from the genome by using fusion PCR.

Luciferase reporter assay

To construct a luciferase reporter plasmid, we purchased pGL3-basic vector (Promega, Madison, USA) and inserted the human wild type or mutant type of AGT 3`UTR sequences into pGL3. HC-a cells were seeded in a 96-well plate for 24 h, followed by co-transfection of luciferase reporter vectors and NC or miR-149-5p mimic using Lipofectamine 2000. For normalisation, we used the Renilla luciferase expression from pRL-TK plasmids (Promega, Madison, USA). After 48 h transfection, the luciferase intensity was measured at 560 nm using the Luciferase Reporter Gene Assay Kit (Promega, Madison, USA).

Statistical analysis

All statistical analyses were performed on GraphPad Prism 5. The data from

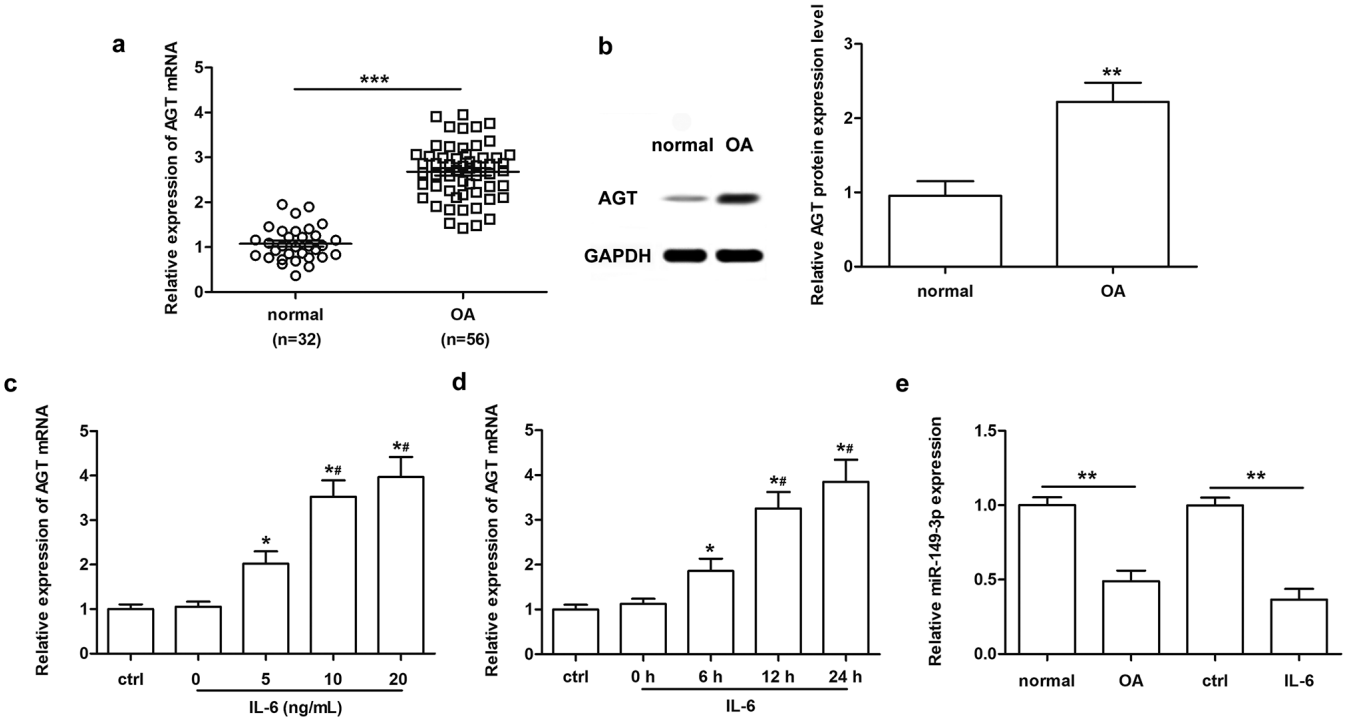

Fig. 1. The expression profile of AGT and miR-149-5p in OA cartilage and IL-6-stimulated chondrocytes.
a: The relative expressions of AGT mRNA in OA cartilage were detected by RT-qPCR. ***$p<0.005$ *vs*. normal. **b**: The visualised and quantified AGT protein level in OA cartilage was measured by western blot. **$p<0.01$ *vs*. normal. **c**: AGT mRNA expression in HC-a cells which were treated with IL-6 at different concentrations (0 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL) for 12 h were detected using RT-qPCR. **d**: After stimulation with 10 ng/mL IL-6 for 0 h, 6 h, 12 h or 24 h, AGT mRNA expression in HC-a cell was measured by RT-qPCR. **e**: Detecting miR-149-5p expression in OA tissue and HC-a cell using RT-qPCR. #$p<0.05$ *vs*. group of 6 h or group of 5 ng/mL. *$p<0.05$ *vs*. ctrl group. Means ± SEM is the form to present the data of at least three repeated experiments.

three individual experiments are expressed as means ± standard error (SEM). Significant differences were analysed using Student's *t*-test or one-way ANOVA. A *p*-value less than 0.05 was considered statistically significant.

Results

AGT was upregulated in OA cartilage tissues and IL-6-stimulated chondrocytes, while miR-149-5p was downregulated

To explore the functions of AGT and miR-149-5p in chondrocytes during OA progression, we collected cartilage from 56 OA patients and 32 healthy volunteers to evaluate their expression patterns. RT-qPCR and western blot indicated that both mRNA and protein expression levels of AGT were upregulated in OA cartilage tissues compared with normal cartilage (Fig. 1a and 1b). The expression of AGT in HC-a cells was also elevated by IL-6 in both dose- and time-dependent manners (Fig. 1c-d). Besides, miR-149-5p was a significantly down-regulated miRNA in OA cartilage tissues (Fig. 1e).

AGT knockdown inhibited inflammatory responses in chondrocytes induced by IL-6 stimulation by blocking RAS

To analyse the potential role of AGT in the development of OA, we transfected IL-6-induced HC-a cells with siAGT or treated them with ACEI. The transfection efficiency experiment showed that with increasing siAGT transfection concentration, the IL-6-induced AGT upregulation was gradually inhibited (Fig. 2a). Both mRNA and protein expression levels of ANG II and AT1R were enhanced by IL-6 and efficiently inhibited by siAGT and ACEI (Fig. 2b-d). The levels of cytokines, IL-1β, MMP-13 and nitrite, all of which are the hallmarks of OA inflammation in human chondrocytes, were significantly increased by IL-6 stimulation, while siAGT transfection and ACEI treatment suppressed these elevations (Fig. 2e-g). Furthermore, siAGT and ACEI inhibited IL-1β and MMP-13 mRNA level changes induced by IL-6 (Fig. 2h). Overall, these consequences demonstrated that AGT participated in the regulation of inflammatory responses of OA chondrocytes via modulating RAS.

MiR-149-5p participated in the regulation of the OA chondrocytic inflammatory responses via directly binding to AGT

We submitted AGT and miR-149-5p to an online bioinformatics prediction platform, TargetScan, and predicted the potential binding. As shown in Figure 3a, miR-149-5p bond to the 3`UTR of AGT at the specific binding motif, GAGCCAG (725 bp ~731 bp). We introduced 4 consecutive mutations at the binding region between AGT and miR-149-5p by using fusion PCR. Thus, we obtained the mutated AGT 3`UTR (Fig. 3b). The luciferase reporter assay indicated that miR-149-5p mimic transfection decreased the luciferase activity of the wild type but not the mutant 3`UTR of AGT (Fig. 3c).

After establishing the binding relationship between miR-149-5p and AGT, it was necessary to validate the role of miR-149-5p in regulating AGT-mediated chondrocytic inflammatory

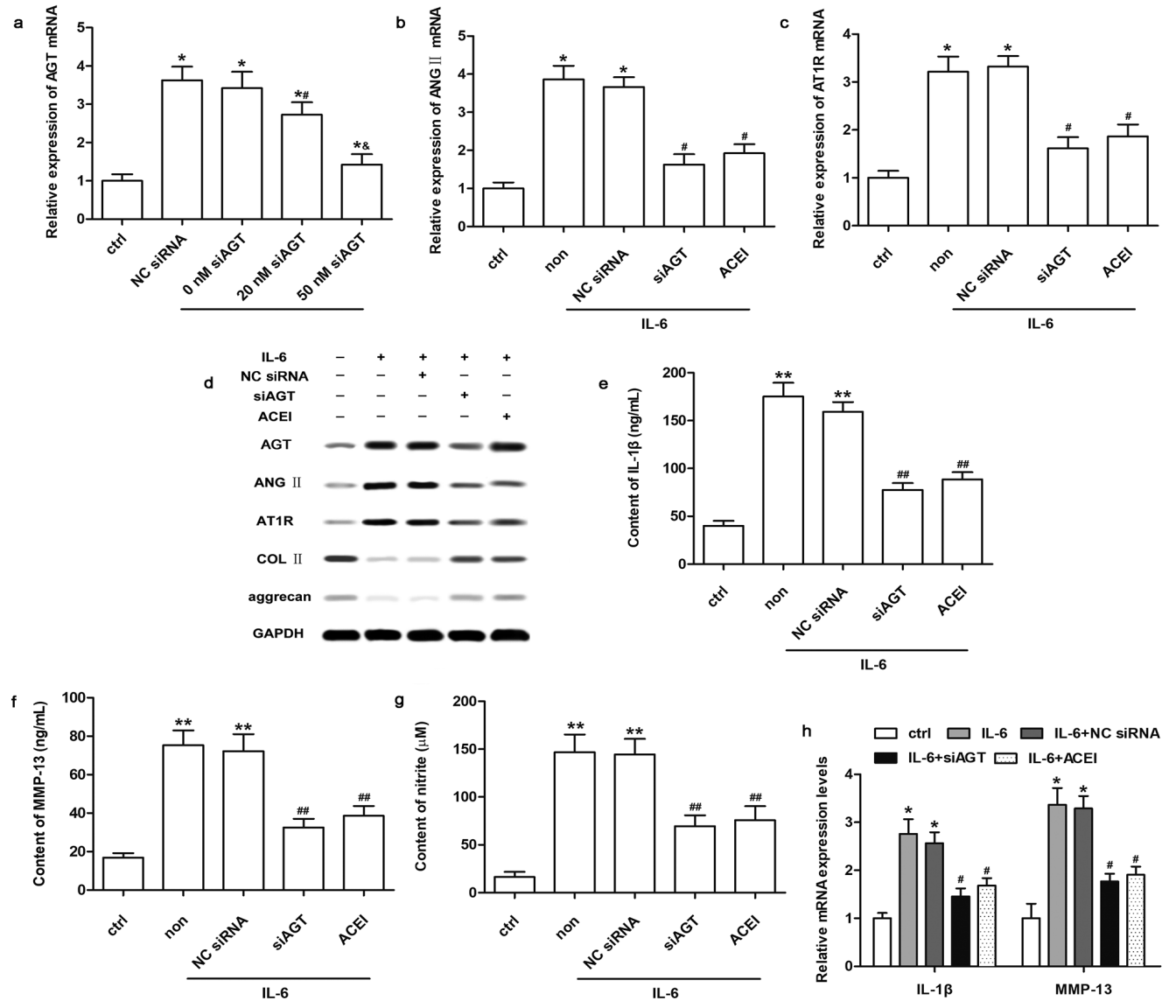

Fig. 2. AGT affected IL-6-stimulated inflammatory responses in chondrocytes and RAS system.
a: After IL-6 stimulation, the expressions of AGT in NC siRNA, 0 nM siAGT, 20 nM siAGT or 50 nM siAGT transfected cells were detected by RT-qPCR; $^{*}p<0.05$ *vs*. ctrl gourp; $^{\#}p<0.05$ *vs*. NC siRNA group; $^{\&}p<0.05$ *vs*. 20 nM siAGT group. HC-a cells were transfected with siAGT or treated with ACEI followed by IL-6 stimulation; the mRNA expressions of ANG II (**b**), AT1R (**c**), IL-1β and MMP-13 (**h**) were measured using RT-qPCR, while western blot showed the protein levels of ANG II and AT1R (**d**). Elisa was used to determine the contents of IL-1β (**e**) and MMP-13 (**f**). Nitrite levels were detected with Griess reagent (**g**).
Data are presented as means ± SEM from three replicates. $^{*}p<0.05$ *vs*. ctrl. $^{\#}p<0.05$ *vs*. IL-6 group. $^{**}p<0.01$ *vs*. ctrl or normal group. $^{\#\#}p<0.01$ *vs*. IL-6 group.

response. Through transfection assays, we found that the miR-149-5p mimic reversed the inhibitory effect of IL-6 on the expression of miR-149-5p, while the miR-149-5p inhibitor aggravated this effect (Fig. 3d). Besides, the miR-149-5p mimic depressed IL-6-induced mRNA and protein upregulations of AGT, ANG II and AT1R. Comparatively, the miR-149-5p inhibitor presented opposite effects; intriguingly, AGT overexpression almost counteracted the effects of the miR-149-5p mimic (Fig. 3e-h). Compared with the above results, transfections of miR-149-5p mimic and inhibitor, as well as pcDNA-AGT, had consistent effects on the expressions and contents of IL-1β and MMP-13, and the level of nitrite (Fig. 3i-l). Overall, our data suggested that miR-149-5p participated in the regulation of chondrocytic inflammation responses in OA via directly binding to AGT.

AGT regulated IL-6-induced chondrocytic inflammation responses via JAK2/STAT3 pathway

In order to further explore the molecular mechanism of AGT regulating OA inflammation, we detected JAK2/STAT3 activity in OA cartilage tissue samples. As shown in Figure 4a, significant phosphorylation of JAK2 and STAT3 were detected in OA cartilage tissues compared with normal tissues. We also found that JAK2 and STAT3 were activated by IL-6 in HC-a cells; however, both siAGT and the JAK/STAT inhibitor (WP1066) reversed the enhancement of p-JAK2 and p-STAT3 levels induced by IL-6 (Fig. 4b). Notably, WP1066 had no effects

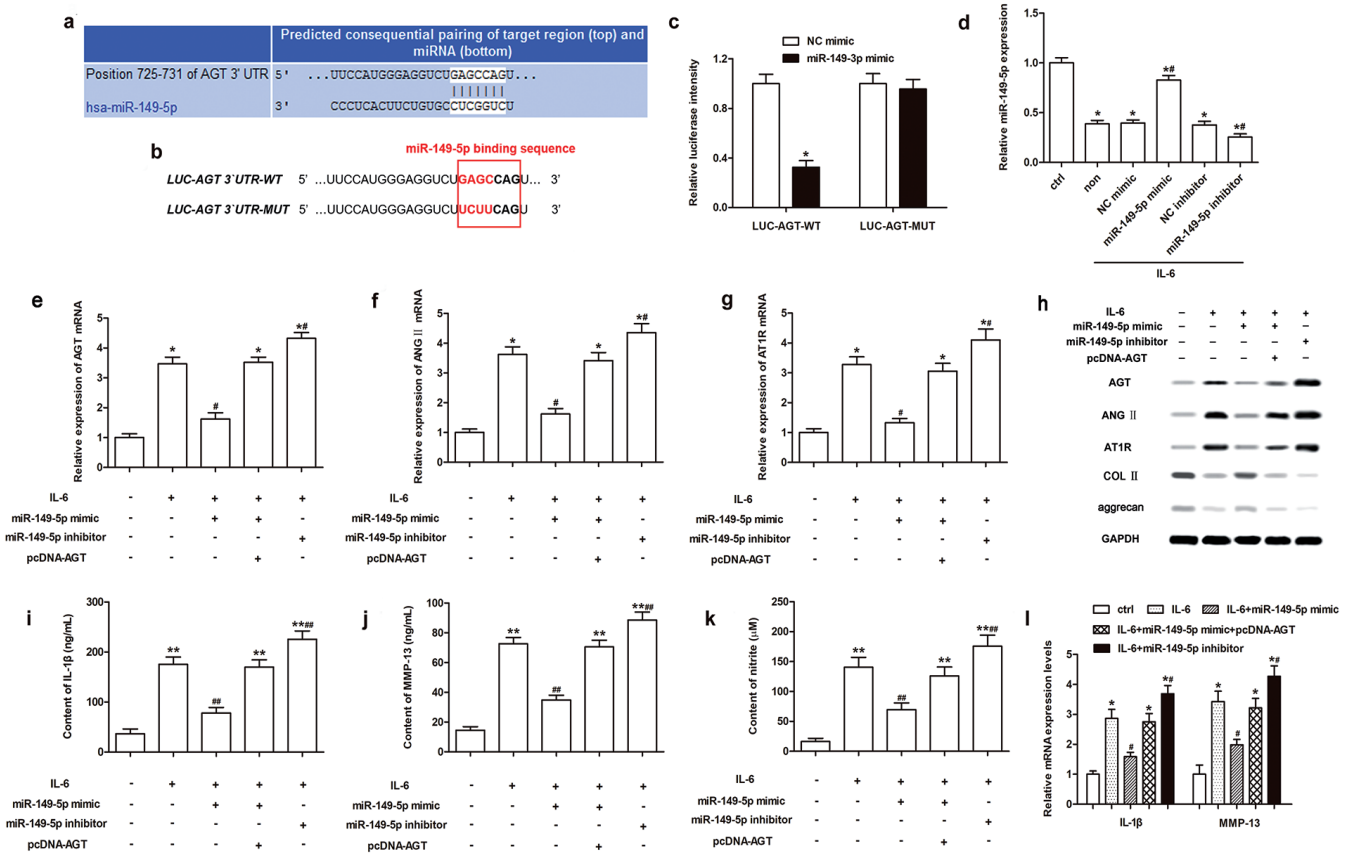

Fig. 3. MiR-149-5p directly bond to the 3`UTR of AGT.
a: TargetScan predicted the binding between miR-149-5p and AGT. **b**: Mutations were introduced into the 3`UTR of AGT using Fusion PCR and specific primers. **c**: Luciferase reporter assay presented the luciferase intensity of AGT 3`UTR with miR-149-5p or NC mimic. $^{*}p<0.05$ *vs*. NC mimic. HC-a cells were transfected with miR-149-5p mimic or miR-149-5p inhibitor with or without pcDNA-AGT, followed by IL-6 treatment. Relative expressions of miR-149-5p (**d**), AGT (**e**), ANG II (**f**), AT1R (**g**), IL-1β and MMP-13 (**l**) were detected by RT-qPCR. The fold changes of protein levels, AGT, ANG II and AT1R (**h**), were measured using western blot. Elisa assay was conducted to measure the contents of IL-1β (**i**) and MMP-13 (**j**). **k**: Nitrite content was detected with Griess reagent. All data are expressed as mean ± SEM. $^{*}p<0.05$ *vs*. ctrl; $^{\#}p<0.05$ *vs*. non-treated IL-6 group; $^{**}p<0.01$ *vs*. ctrl; $^{\#\#}p<0.01$ *vs*. IL-6 group.

on the IL-6-induced upregulations of AGT, ANG II and AT1R, while AGT overexpression exacerbated the IL-6-indcued increase of both mRNA and protein expressions of these factors (Fig. 4c-d). Nevertheless, WP1066 suppressed IL-6-induced elevation of IL-1β, MMP-13 and nitrite contents, which were counteracted by AGT overexpression (Fig. 4e-g). WP1066 and pcDNA-AGT exerted similar effects on the relative levels of IL-1β and MMP-13 (Fig. 4h). That was to say, AGT regulated IL-6-induced chondrocytic inflammation responses via JAK2/STAT3 pathway.

Discussion

Although OA was validated as a degenerative inflammatory disease that occurred in the joints, especially cartilage (1), there was few evidence elucidated its pathogenesis. A previous study showed that AGT was a component of RAS which was involved in the regulations of many cardiovascular functions, such as systemic blood pressure (26). Also, the elevated AGT expression was detected in the intra-renal of hypertension patients (27). Ryousuke Satou and colleagues revealed that the augmented AGT expression accelerated ANG I and ANG II formation and stimulated AT1R transport function, thus to exacerbate hypertension development and progression (28). Recent studies documented that RAS was associated with OA pathology and the aberrant expression of RAS members was implicated in chondrocytes differentiation and OA (15, 29, 30). More importantly, researchers also discovered that AGT was upregulated in knee OA tissues (21). In this study, the expression of AGT was elevated in OA cartilage tissue. Moreover, we found that AGT overexpression increased the expression of ANG II and AT1R, and aggravated the chondrocytic inflammatory responses under IL-6 stimulation.

Interestingly, our study validated that miR-149-5p could directly bind to the 3`UTR of AGT and regulate IL-6-stimulated inflammatory responses in chondrocyte. Previously, most studies indicated that miR-149-5p regulated the proliferation, migration and invasion of cancer cells (31, 32). Recently, scientists found that miR-149-5p was downregulated in OA chondrocytes and was associated with the increased levels of pro-inflammatory cytokines (25, 33). This finding was also validated in our research. Moreover, miR-149 targeted and regulated TAK1, thus to inhibit the IL-1β-triggered inflammatory response of chondrocytes in OA (34). Wang *et*

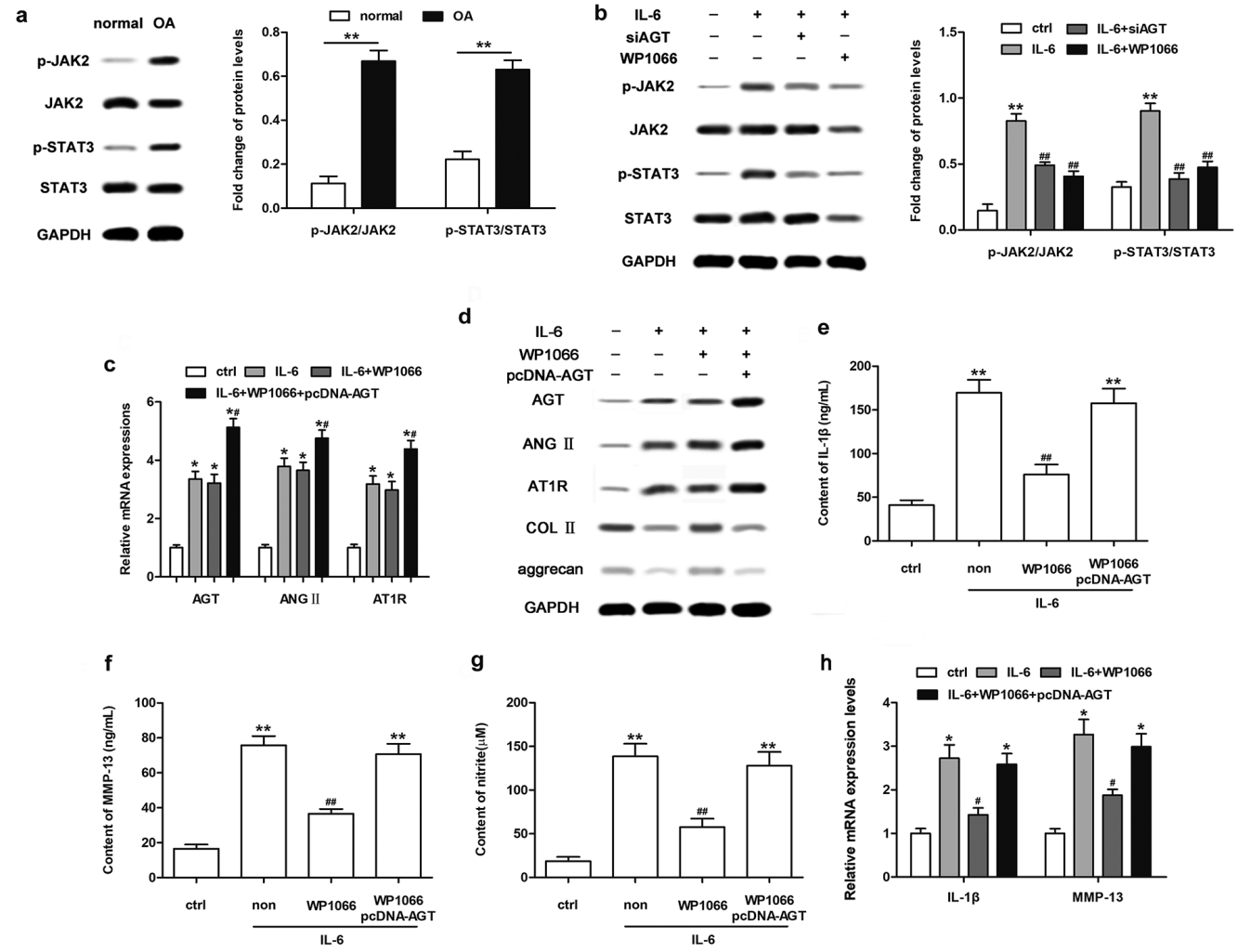

Fig. 4. AGT regulated IL-6-stimulated chondrocytic inflammation responses via JAK2/STAT3 pathway.
a: The quantified and visualised fold changes of p-JAK2/JAK and p-STAT3/STAT3 in OA and normal cartilage tissues were detected using western blot. $^{**}p<0.01$ *vs*. normal group. **b**: After transfected with siAGT or treated with WP1066, HC-a cells were stimulated with IL-6; the fold change of protein level was detected by western blot. Cells were treated by WP1066 with or without pcDNA-AGT transfection, followed by IL-6 stimulation. RT-qPCR showed the mRNA expressions of AGT, ANG II and AT1R (**c**), IL-1β and MMP-13 (**h**). Western blot was conducted to detect the relative protein levels of AGT, ANG II and AT1R (**d**). Elisa assay indicated the changes of IL-1β (**e**) and MMP-13 (**f**) contents. **g**: The NO level were measured by nitrite estimation using Griess reagent. All data were presented as means ± SEM of three individual experiments. $^{*}p<0.05$ *vs*. ctrl; $^{\#}p<0.05$ *vs*. IL-6 group; $^{**}p<0.01$ *vs*. ctrl and $^{\#\#}p<0.01$ *vs*. IL-6 group.

al. suggested that miR-149 could also mediate OA development by targeting FUT1 (35).

In recent years, JAK2/STAT3 pathway emerged as an intracellular signalling with relevance to multiple cellular processes, including apoptosis and inflammatory reaction (36). Also, JAK/STAT signalling played a key role in the pathological process of arthritis (37). Zhang and colleagues conducted a study using an OA model and demonstrated that activating JAK2/STAT3 signalling was an important pathway for leptin to inhibit chondrocyte viability and induce apoptosis (38). An *in vitro* study validated that IL-1β stimulation promoted MMP-13 expression in chondrocytes via JAK/STAT pathway (39). Similarly, our present study indicated that JAK2/STAT3 signalling was the downstream pathway of AGT in regulating IL-6-induced inflammation responses of chondrocytes.

In conclusion, AGT presented the pro-inflammatory ability in IL-6-stimulated chondrocytes via regulating JAK2/STAT3 signalling. MiR-149-5p, a downregulated miRNA in OA cartilage tissues, also modulated the IL-6-induced inflammatory responses of chondrocytes, due to its direct binding with AGT. Our study may provide promising therapeutic targets for OA therapy.

References

1. ARDEN N, NEVITT MC: Osteoarthritis: epidemiology. *Best Pract Res Clin Rheumatol* 2006; 20: 3-25.
2. ZHANG M, THELEMAN JL, LYGRISSE KA, WANG J: Epigenetic mechanisms underlying the aging of articular cartilage and osteoarthritis. *Gerontology* 2019: 1-10.
3. XIE J, HUANG Z, YU X, ZHOU L, PEI F: Clinical implications of macrophage dysfunction in the development of osteoarthritis of the knee. *Cytokine Growth Factor Rev* 2019; 46: 36-44.
4. CROSS M, SMITH E, HOY D *et al*.: The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. *Ann Rheum Dis* 2014; 73: 1323-30.
5. WALLACE IJ, WORTHINGTON S, FELSON DT *et al*.: Knee osteoarthritis has doubled in prevalence since the mid-20th century. *Proc Natl Acad Sci USA* 2017; 114: 9332-6.

6. GOLDRING MB: Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. *Best Pract Res Clin Rheumatol* 2006; 20: 1003-25.
7. HUNTER DJ, BIERMA-ZEINSTRA S: Osteoarthritis. *Lancet* 2019; 393: 1745-59.
8. KUTTAPITIYA A, ASSI L, LAING K *et al*.: Microarray analysis of bone marrow lesions in osteoarthritis demonstrates upregulation of genes implicated in osteochondral turnover, neurogenesis and inflammation. *Ann Rheum Dis* 2017; 76: 1764-73.
9. BONNET CS, WALSH DA: Osteoarthritis, angiogenesis and inflammation. *Rheumatology* 2005; 44: 7-16.
10. LI H, LI L, MIN J *et al*.: Levels of metalloproteinase (MMP-3, MMP-9), NF-kappaB ligand (RANKL), and nitric oxide (NO) in peripheral blood of osteoarthritis (OA) patients. *Clin Lab* 2012; 58: 755-62.
11. WANG D, QIAO J, ZHAO X, CHEN T, GUAN D: Thymoquinone inhibits IL-1beta-induced inflammation in human osteoarthritis chondrocytes by suppressing NF-kappaB and MAPKs signaling pathway. *Inflammation* 2015; 38: 2235-41.
12. QU Y, ZHOU L, WANG C: Mangiferin inhibits IL-1beta-induced inflammatory response by activating PPAR-gamma in human osteoarthritis chondrocytes. *Inflammation* 2017; 40: 52-7.
13. CASTROP H, HOCHERL K, KURTZ A *et al*.: Physiology of kidney renin. *Physiol Rev* 2010; 90: 607-73.
14. KUNIKULLAYA UK, ANANTHAKRISHNAN V, GOTURU J: Robert Tigerstedt and the discovery of renin - a revisit. *Int J Cardiol* 2012; 158: 1-5.
15. YAMAGISHI K, TSUKAMOTO I, AKAGI M: Activation of the renin-angiotensin system introduces osteoarthritis. *Osteoarthritis Cartilage* 2015; 23: A320-A1.
16. YONGTAO Z, KUNZHENG W, JINGJING Z *et al*.: Glucocorticoids activate the local renin-angiotensin system in bone: possible mechanism for glucocorticoid-induced osteoporosis. *Endocrine* 2014; 47: 598-608.
17. KAWAKAMI Y, MATSUO K, MURATA M *et al*.: Expression of angiotensin II receptor-1 in human articular chondrocytes. *Arthritis* 2012; 2012: 648537.
18. ÇOBANKARA V, ÖZTÜRK MA, KIRAZ S *et al*.: Renin and angiotensin-converting enzyme (ACE) as active components of the local synovial renin-angiotensin system in rheumatoid arthritis. *Rheumatology Int* 2005; 25: 285-91.
19. BERNSTEIN KE, GIANI JF, SHEN XZ, GONZALEZ-VILLALOBOS RA: Renal angiotensin-converting enzyme and blood pressure control. *Curr Opin Nephrol Hypertens* 2014; 23: 106.
20. PURKAIT P, HALDER K, THAKUR S *et al*.: Association of angiotensinogen gene SNPs and haplotypes with risk of hypertension in eastern Indian population. *Clin Hypertens* 2017; 23: 12.
21. LI M, ZHI L, ZHANG Z, BIAN W, QIU Y: Identification of potential target genes associated with the pathogenesis of osteoarthritis using microarray based analysis. *Mol Med Rep* 2017; 16: 2799-806.
22. YATES LA, NORBURY CJ, GILBERT RJ: The long and short of microRNA. *Cell* 2013; 153: 516-9.
23. LIN S, GREGORY RI: MicroRNA biogenesis pathways in cancer. *Nat Rev Cancer* 2015; 15: 321-33.
24. GU R, LIU N, LUO S *et al*.: MicroRNA-9 regulates the development of knee osteoarthritis through the NF-kappaB1 pathway in chondrocytes. *Medicine* 2016; 95: e4315.
25. WANG X, NING Y, ZHOU B *et al*.: Integrated bioinformatics analysis of the osteoarthritis-associated microRNA expression signature. *Mol Med Rep* 2018; 17: 1833-8.
26. DZIMIRI N, AL-NAJAI M, ELHAWARI S *et al*.: The interaction of Agt gene polymorphism with cardiovascular risk traits in atherosclerosis: *Arterioscl Throm Vas* 2012; 32; A524.
27. NAVAR LG: Translational studies on augmentation of intratubular renin–angiotensin system in hypertension. *Kidney Int Suppl* 2013; 3: 321-5.
28. SATOU R, SHAO W, NAVAR LG: Role of stimulated intrarenal angiotensinogen in hypertension. *Ther Adv Cardiovasc Dis* 2015; 9: 181-90.
29. TSUKAMOTO I, INOUE S, TERAMURA T *et al*.: Activating types 1 and 2 angiotensin II receptors modulate the hypertrophic differentiation of chondrocytes. *FEBS Open Bio* 2013; 3: 279-84.
30. TSUKAMOTO I, AKAGI M, INOUE S *et al*.: Expressions of local renin-angiotensin system components in chondrocytes. *Eur J Histochem* 2014; 58: 2387.
31. JIN L, LI Y, LIU J *et al*.: Tumor suppressor miR-149-5p is associated with cellular migration, proliferation and apoptosis in renal cell carcinoma. *Mol Med Rep* 2016; 13: 5386-92.
32. SINGH S, EBRON JS, SHANKAR E *et al*.: Regulation of androgen signaling axis and tumor suppressive function of miR-149-5p in prostate cancer. *Cancer Res* 2017; 77; 2531.
33. SANTINI P, POLITI L, VEDOVA PD, SCANDURRA R, SCOTTO D'ABUSCO A: The inflammatory circuitry of miR-149 as a pathological mechanism in osteoarthritis. *Rheumatology Int* 2014; 34: 711-6.
34. CHEN Q, WU S, WU Y, CHEN L, PANG Q: MiR-149 suppresses the inflammatory response of chondrocytes in osteoarthritis by down-regulating the activation of TAK1/NF-κB. *Biomed Pharmacother* 2018; 101: 763.
35. WANG Z, HU J, PAN Y *et al*.: miR-140-5p/miR-149 affects chondrocyte proliferation, apoptosis, and autophagy by targeting FUT1 in osteoarthritis. *Inflammation* 2018; 41: 1-13.
36. JIA L, WANG F, GU X *et al*.: Propofol postconditioning attenuates hippocampus ischemia-reperfusion injury via modulating JAK2/STAT3 pathway in rats after autogenous orthotropic liver transplantation. *Brain Res* 2017; 1657: 202-7.
37. MALEMUD CJ: Negative regulators of JAK/STAT signaling in rheumatoid arthritis and osteoarthritis. *Int J Mol Sci* 2017; 18: 484.
38. ZHANG ZM, SHEN C, LI H *et al*.: Leptin induces the apoptosis of chondrocytes in an in vitro model of osteoarthritis via the JAK2-STAT3 signaling pathway. *Mol Med Rep* 2016; 13: 3684.
39. LIM H, KIM HP: Matrix metalloproteinase-13 expression in IL-1β-treated chondrocytes by activation of the p38 MAPK/c-Fos/AP-1 and JAK/STAT pathways. *Arch Pharm Res* 2011; 34: 109-17.