LncRNA PVT1 Regulates Biological Function of Osteoarthritis Cells by Regulating miR-497/AKT3 Axis

Peng Chen (ttoxehs@gmail.com)
Chongqing Emergency Medical Center, Chongqing University Central Hospital, Chongqing, 400014, China

Ying Zhang
Chongqing Emergency Medical Center, Chongqing University Central Hospital, Chongqing, 400014, China

Research Article

Keywords: PVT1, miR-497, AKT3, osteoarthritis, chondrocyte

Posted Date: November 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1035573/v1

Abstract

Objective

There is growing evidence that lncRNA plays a role in the progression of many diseases, including osteoarthritis (OA). However, the role of lncRNA PVT1 in OA is still unclear. Thus, the present study aims to explore the function of PVT1 in the OA process, and analyze the possible mechanisms thereof.

Methods

PVT1 expression in articular cartilage tissue of OA patients and non-OA patients was evaluated. PVT1, miR-497 and AKT3 in IL-1β induced chondrocytes were knocked down or elevated to determine the proliferation and apoptosis of treated chondrocytes. And the changes of AKT3 and extracellular matrix (ECM) related factors (aggrecan, collagen Type II and MMP-9) were detected.

Results

PVT1 was elevated in OA cartilage tissue and chondrocytes induced by IL-1. Cell proliferation ability was enhanced while apoptosis rate was declined after PVT1 knock down and miR-497 elevation. In addition, aggrecan and collagen Type II levels reduced in IL-1β induced chondrocytes, while MMP-9 level elevated. Dual luciferase reporter confirmed that PVT1 and AKT3 interacted with miR-497, respectively. Rescue experiments revealed that increasing AKT3 or knocking down miR-497 could reverse the impacts of knocking down PVT1 on IL-1β-induced chondrocytes.

Conclusion

PVT1 is up-regulated in OA, and knock down PVT1 can decrease the effects of IL-1βon proliferation, apoptosis, and ECM degradation of chondrocyte through regulating miR-497/AKT3 axis.

1 Introduction

Osteoarthritis (OA), the most common degenerative disease of joints, is characterized by articular cartilage degeneration, subchondral osteosclerosis and osteophyte formation[1]. The main symptoms of OA include joint pain, joint stiffness and decreased range of motion, seriously affecting quality of life[2]. At present, OA is considered an incurable disease, and is mainly treated clinically for the purpose of improving joint function and pain relief[3]. Therefore, it is urgent to elucidate the potential mechanism of OA pathogenesis to find promising therapeutic targets.

Long-chain non-coding RNAs (lncRNAs) are a group of non-coding RNAs with a length of more than 200 nucleotides, which can be involved in and regulate numerous biological processes, such as cell

differentiation, proliferation, autophagy and apoptosis [4–6]. In recent years, intensive research has revealed the important roles of lncRNA in the occurrence and progression of numerous diseases. For example, it was found that lncRNA DLEU1 has elevated levels in colorectal cancer and promotes the malignant growth of cancer cells[7]. Also, a high expression of lncRNA UCA1, acting as carcinogenic factor, was associated with poor prognosis for OA patients[8]. The lncRNA SNHG5 was shown to promote the proliferation and migration of chondrocytes through the miR-26a/SOX2 axis, thus participating in the progression of OA[9]. The lncRNA PVT1 is located at the 8q24.21 region of the human chromosome. It is one of lncRNAs extensively studied in recent years, as it is considered a cancer-promoting gene and a potential therapeutic target for tumors[10, 11]. Studies in the past few years have demonstrated that PVT1 is elevated in OA, and it can regulate chondrocyte apoptosis via acting as a sponge of miR-488-3p[12]. A further study supports that PVT1 has higher levels in cartilage tissue of OA patients; the inhibition of its expression can reduce IL-1β-induced chondrocyte injury[13]. The studies above indicate that PVT1 is expected to become an important target for OA therapy. Therefore, further research is necessary to improve our understanding of the exact role PVT1 plays in the mechanism and progression of OA.

Herein, we detected PVT1 expression in articular cartilage tissues of OA and non-OA patients, and designed in vitro experiments to analyze its role and mechanism of action in OA progression

2 Materials And Methods

2.1 Patients

The cross-sectional study was carried out with the authorization of the Hospital Medical Ethics Association. The study protocols were performed in accordance with the ethical tenets of the Declaration of Helsinki. After obtaining informed consent, we acquired articular cartilage specimens from 24 patients with the presence of knee osteoarthritis diagnosed according to the American College of Rheumatology classification criteria at Department of Spine and Osteopathy Ward, Yuebei People's Hospital from February 2016 to June 2018. All patients who received intra-articular injection or with psoriasis arthritis or rheumatoid arthritis were excluded.A total of 20 articular cartilage tissue samples from non-OA patients who received tibial plateau fractures, and postoperative pathology was not suggestive of arthritis and rheumatoid arthritis were applied as controls for validation. Fresh articular cartilage tissue samples of the patients were stored in a refrigerator at -80℃.

2.2 Cell processing

2.2.1 Chondrocyte source and culture

The ATDC5 chondrocytes were purchased from ATCC cell bank. Cells were placed in DMEM (Gibco, USA) medium comprising 10% fetal bovine serum (FBS, Gibco, USA), and The medium was then cultured in an incubator with 5% CO_2 under at 37℃. Cells at the logarithmic growth stage were placed in 10 μg/L IL-1β medium containing 10 μg/L to simulate OA *in vitro*.

2.2.2 Cell grouping and processing

A pcDNA 3.1 plasmid was used as vector to establish the PVT1 inhibitory plasmid (si-PVT1), the miR-497 over-expression plasmid (miR-497-mimics) and the AKT3 over-expression plasmid (sh-AKT3). Cells were transfected with si-NC was used as the control of for lncRNA, and miR-NC was used as the control of for miR-497. SubsequentlyAccordingly, different plasmids were transfected into chondrocytes induced by IL-1β by (Invitrogen™, USA) and cultured for 24 hours.

2.3 qRT-PCR

Tissues and cells to be tested were harvested for total RNA extraction using TRIzol kit (Invitrogen, USA); UV spectrophotometer and agarose gel electrophoresis were applied for the determination of RNA purity, concentration and integrity. Qualified RNA (2 μg) was reversely transcribed into cDNA using a reverse transcription kit (Invitrogen, USA), which in turn was amplified by PCR using PrimeScript RT Master Mix kit (Takara Bio, Japan), consisting of the following: 10 μL SYBR qPCR Mix, 0.8 μL upstream primers, 0.8 μL downstream primers, 2 μL cDNA, 0.4 μL 50×ROX reference dye; and distilled water to supplement the solutions to 20 μL. Conditions for the PCR reaction were as follows: 95℃ for 60 s, 95℃ for 30 s, 60℃ for 40 s with 40 cycles in total; $2^{-\triangle\triangle ct}$ was applied for calculation[14].

2.4 Western blot (WB)

Total protein was extracted by RIPA (Thermo Scientific, USA). Bicinchoninic acid (Thermo Fisher, USA) was applied to determine protein concentration. A 40 μg sample was taken for 10% polyacrylamide gel electrophoresis (120 v) before the protein was transferred to PVDF membrane (Life Technologies, USA) and blocked with 5% skim milk powder for 2 hours at 37℃. Samples were then incubated with Aggrecan (1:1500), collagen Type II (1:1500), MMP-9 (1:1500), AKT3 (1:1500) and β-catenin primary antibody (1:1500) (Abcam, USA) overnight at 4℃. Following the removal of primary antibody, horseradish peroxidase labeled goat anti rabbit secondary antibody (1:4000, Abcam, USA) was added before two-hour incubation at 37℃, then rinsed for 4×5 min each with PBS. The electrochemiluminescence of samples was measured in a darkroom with the ECL method. Subsequently, grayscale analysis was conducted for the calculation of relative expression level of the target protein.

2.5 Cell proliferation detection

An MTT kit (Beijing Baiaolaibo Technology Co., Ltd) was used for the detection of cell metabolic activity. Cells were transferred to 96-well plates (density: 5000 cells/well), followed by the addition of 20 μL MTT solution into each well at different times for incubation at 37℃ for 4 hours. Subsequently, wells were incubated for 10 min with 150 μL dimethyl sulfoxide. Finally, absorbance was assessed by a microplate reader at 490 nm wavelength.

2.6 Cell apoptosis detection

Cell digestion was performed with 0.25% trypsin and cells were configured into 1×10^6 /mL suspension. Ten μL AnnexinV-FITC/PI (Shanghai Yeasen Biotechnology Co., Ltd) was added successively to each well

for incubation in a darkroom at 37°C for 5 min. Flow cytometry (FCM) BD FACSCanto™ II was applied for analysis and calculation of apoptosis rate.

2.7 Dual luciferase reporter

The starBase3.0 and Targetscan7.2 biological prediction websites were used to find potential target genes of PVT1 and miR-497, whereas a Lipofectamine™ 2000 kit was used to establish PVT1-3'UTR wild type (Wt), PVT1-3'UTR mutant (Mut), AKT3-3'UTR Wt and AKT3-3'UTR Wt, respectively. Sections of RNA were transferred to the downstream of luciferase reporter gene to sequence and identify the constructed plasmid, and then transfected to HEK 293T cells (ATCC) together with miR-497-mimics and miR-NC. Changes in luciferase activity were detected by the aid of a dual luciferase reporter assay kit (Solarbio, Bejing, China).

2.8 Statistical methods

All sample data were obtained from three separate experiments. Data analysis was conducted utilizing the SPSS18.0 software package, while image rendering was done by GraphPad 7. Data were expressed in the form of mean ± standard deviation (Meas±SD). Comparisons between two groups were made by an independent sample t test, intergroup comparisons were performed by one-way analysis of variance (ANOVA), an LSD-t test was applied for pairwise comparison, while repeated measurement ANOVA was utilized for expression at multiple time points. Bonferroni correction was utilized for Back testing. Statistical differences were indicated in all cases, where $P < 0.05$.

3 Results

3.1 PVT1 is elevated in OA cartilage tissue and IL-1β-induced chondrocytes

We detected the lncRNA PVT1 level between 24 OA patients and 24 non-OA patients.There were no significant differences in age, gender, weight, height and other sociodemographic data between the two groups. Detection by qRT-PCR showed that PVT1 expression in the cartilage tissue of the OA patients group was much higher than that of the non-OA OA patients group(Fig. 1A). Furthermore, PVT1 expression was elevated in IL-1β-induced chondrocytes (Fig. 1B).

3.2 Knockdown of PVT1 in IL-1β-induced chondrocytes results in enhanced cell proliferation ability, decreased cell apoptotic rate and altered extracellular matrix (ECM) related genes

To explore the role of PVT1 in OA development, we knocked down PVT1 expression in IL-1β-induced chondrocytes and observed changes in cell proliferation, apoptosis and inflammatory factor levels. Results for MTT and FCM showed that the proliferation ability of IL-1β-induced chondrocytes decreased, while cell apoptotic rate increased significantly compared to normal chondrocytes. When PVT1 was knocked down in IL-1β-induced chondrocytes(Fig. 2A), however, cell proliferation ability was enhanced(Fig. 2B) and apoptotic rate declined significantly(Fig. 2C). At the same time, ECM related genes

were detected, aggrecan and Collagen Type II levels decreased, and MMP-9 increased in chondrocytes after IL-1β induction (Fig. 2D). Knocking down PVT1 in chondrocytes was able to reduce the effects of IL-1β on the three proteins. This suggests that PVT1 may have a marked effect on the progress of OA .

3.3 The interplay of miR-497 and PVT1

The function of lncRNA is realized via the regulation of miR expression. In order to understand how PVT1 regulates the biological behavior of chondrocytes, we applied prediction by starBase 2.0. We found targeted binding sites between miR-497 and PVT1 (Fig. 3A). The expression of miR-497 in chondrocytes was lowered after IL-1β induction (Fig. 3B). Subsequently, we explored the relationship between miR-497 and PVT1. Changes in miR-497 levels in chondrocytes induced by IL-1β were detected after PVT1 knockdown, indicating that PVT1 knockdown could increase miR-497 in cells. In addition, we further analyzed the relationship between miR-497 and PVT1 via a dual luciferase reporter. According to results, the transfection of miR-497 mimics were able to inhibit PVT1-3'UTR Wt luciferase activity without affecting PVT1-3'UTR Mut luciferase activity, suggesting that an interaction between miR-497 and PVT1 took place (Fig. 3C).

3.4 The function of miR-497 is the opposite of PVT1

In this section, we explore the function of miR-497 in the progression of OA. Mimics of miR-NC and miR-497 were transfected separately into chondrocytes induced by IL-1β, which resulted in promoted cell proliferation (Fig. 4B) and reduced apoptotic rate (Fig. 4C) in cells transfected with miR-497 mimics. Moreover, aggrecan and collagen Type II increased, whereas MMP-9 decreased in cells (Fig. 4D). These indicate that the role of miR-497 in OA is the exact opposite of PVT1.

3.5 The role of miR-497 through the negative regulation of AKT3

A potential target gene of miR-497 was predicted to be AKT3 (Fig. 5A). It was revealed by WB that AKT3 was elevated in IL-1β induced chondrocytes and dropped in cells transfected with miR-497 mimics (Fig. 5B). The association between miR-497 and AKT3 was analyzed by a dual luciferase reporter. It could be seen that miR-497 mimics could inhibit AKT3-3'UTR Wt luciferase activity without causing great changes in AKT3-3'UTR Mut miR-497 luciferase activity (Fig. 5C). Next, we transfected IL-1β-induced chondrocytes with miR-497-mimics and sh-AKT3. Observing the proliferation, apoptosis and ECM related molecular changes of OA chondrocytes, it was found that the effect of miR-497 mimics was weakened by sh-AKT3, indicating that miR-497 could act through the negative regulation of AKT3 (Fig. 5D,Fig. 5D and Fig. 5E).

3.6 The regulation of PVT1 in chondrocytes depends on the modulation of the miR-497/AKT3 axis

To further explore the regulatory mechanism between PVT1 and miR-497 or AKT3, we transfected a miR-497 inhibitor and sh-AKT3 separately to OA chondrocytes transfected with si-PVT1. Cell proliferation, apoptosis and changes in ECM related molecules were observed. It was seen that transfection with miR-497-inhibitor or sh-AKT3 could reverse the effect of transfection of si-PVT1 on OA chondrocyte proliferation (Fig. 6A), apoptosis (Fig. 6B) and ECM related molecules (Fig. 6C), suggesting that the regulation of PVT1 in chondrocytes depends on the regulation of the miR-497/AKT3 axis.

4 Discussion

Osteoarthritis, a major cause of disability in the elderly, is still lacking effective methods for treatment[15]. In recent years, a growing number of targeted drugs have shown outstanding therapeutic effects in clinical treatment; finding potential therapeutic targets has become one of the important directions of current research. The occurrence of OA is the result of multiple factors, among which a significant element is a decline in chondrocyte numbers[16]. A variety of lncRNAs are involved in the OA development via regulating the biological behavior of chondrocytes, thus lncRNA has received extensive attention for the past few years[10, 17]. The level of PVT1 increases in chondrocytes of patients, hence knocking down PVT1 in IL-1β treated chondrocytes can not only promote cell activity and autophagy, but also inhibit cell apoptosis and inflammatory response[17]. Herein, elevated PVT1 was found in OA cartilage tissue and chondrocytes induced by IL-1β, whereas knocking down PVT1 could promote proliferation and reduce cell apoptosis in these chondrocytes. Cartilage ECM plays a crucial role in maintaining cartilage structure and function[17]. Interleukin-1β can reduce the synthesis of anabolic genes (aggrecan and collagen Type II) and increase the level of catabolic factors (MMP-9), thus intensifying the degradation of chondrocyte ECM[18, 19]. We found that aggrecan and collagen Type II levels lowered, whereas those of MMP-9 elevated in IL-1β-induced chondrocytes, while knocking down PVT1 reduced the impacts of IL-1β on these three proteins. Consequently, PVT1 knockdown may be one of the main methods to treat OA.

Growing evidence shows that lncRNA can function as a molecular sponge of miRNA by combining with it, thereby participating in biological behaviors. For example, PVT1 promotes the proliferation and migration of pancreatic cancer cells through regulating miR-448 as a molecular sponge[20, 21]. Also, MALAT1 can promote the malignant growth of triple negative breast cancer cells through regulating miR-129-5p[22]. Furthermore, PVT1 knockdown inhibits IL-1β-induced chondrocyte injury by regulating the miR-27b-3P/TRAF3 axis[14]. Here, we used biological prediction software and acquired binding sites between PVT1 and miR-497 to investigate the promising mechanism of PVT1 function involved in OA progression; PVT1 knockdown was able to increase miR-497 in IL-1β-induced chondrocytes. The dual luciferase report also showed that an interaction between miR-497 and PVT1 occurred. The RNAs miRNA and lncRNA are also commonly found in the human body, and can be involved in the regulation of a third of all human genes[23]. The imbalance of miRNA, a key regulator of gene expression, is considered to be a major cause of disease. Previous reports have shown that miR-497, which belongs to the miR-15/16/195/424/497 family, is downregulated in IL-1β treated chondrocytes[24]. In the present study, the presence of this downregulation was also confirmed. In addition, the effects of IL-1β on chondrocyte proliferation and

ECM were weakened upon increasing miR-497 expression in IL-1β treated chondrocytes, which indicates that miR-497 and PVT1 are potential therapeutic targets for OA.

Target genes can be regulated by miR by cutting their mRNA and inhibiting protein synthesis, thus preventing gene function[25]. Biological prediction software was used again for prediction to understand how miR-497 participates in OA progression, with targeted binding sites between miR-497 and AKT3. The expression of AKT3 was detected in chondrocytes transfected with miR-497-mimics and miR-NC, while dual luciferase reporter analysis revealed that AKT3 can be negatively regulated by miR-497. The AKT3 protein comes from the AKT serine/threonine kinase family, which can regulate cell signaling and participate in multiple processes in the cell, such as growth, proliferation, differentiation and apoptosis[26, 27]. It is elevated in many inflammatory diseases including OA[28, 29]. Previous studies have revealed that the AKT3 gene can be used as target for miR, enabling miR to indirectly participate in disease progression. For example, miR-29a can act as a tumor suppressor in papillary thyroid carcinoma by the targeted regulation of AKT3[30]. Also, miR-384 can inhibit the proliferation of colorectal cancer cells through the negative regulation of AKT(Wang, et al., 2018). In this study, we transfected IL-1β-induced chondrocytes with miR-497 mimics and then with sh-AKT3. The impacts of miR-497 mimics on the proliferation, apoptosis and ECM related molecules of IL-1β-induced chondrocytes were reversed by sh-AKT3, suggesting that miR-497 can function through the negative regulation of AKT3[25]. At the end of this study, we conducted rescue experiments to further investigate the regulatory mechanism between PVT1 and miR-497/AKT3. It was found that transfection by miR-497 inhibitor or sh-AKT3 could reverse the effects of transfection by si-PVT1 on proliferation, apoptosis and ECM-related molecules of OA chondrocytes. Therefore, we believe that the regulation of PVT1 in chondrocytes depends on the regulation of the miR-497/AKT3 axis.

Although this study confirmed that the knockdown of PVT1 could decrease the effects of IL-1β on proliferation, apoptosis and degradation of chondrocyte ECM through the miR-497/AKT3 axis, it still has some deficiencies. Firstly, for example, no animal experiments have been conducted to analyze the effects of PVT1 on subchondral osteosclerosis and osteophyte formation. Secondly, neither the expression levels of miR-493 and AKT3 in OA cartilage tissue were measured, nor the clinical effects of PVT1, miR-497 and AKT3 in OA were investigated. Moreover, potential mechanisms of PVT1 function other than the miR-497/AKT3 axis have not been explored. The above limitations are expected to be corrected by further experiments in follow-up studies.

5 Conclusion

In summary, it is acknowledged that PVT1 is elevated in OA cartilage tissue and IL-1β-induced chondrocytes, and PVT1 knockdown can reduce the effects of IL-1β on proliferation, apoptosis and degradation of chondrocyte ECM by regulating the miR-497/AKT3 axis.

References

1. Park S, Lee M, Chun CH, et al. The lncRNA, Nespas, Is Associated with Osteoarthritis Progression and Serves as a Potential New Prognostic Biomarker. Cartilage. 2019;10(2):148–156.
2. Sun H, Peng G, Ning X, et al. Emerging roles of long noncoding RNA in chondrogenesis, osteogenesis, and osteoarthritis. Am J Transl Res. 2019;11(1):16–30.
3. Goldring MB, Berenbaum F. Emerging targets in osteoarthritis therapy. Curr Opin Pharmacol. 2015;22:51–63.
4. Xue X, Yang YA, Zhang A, et al. LncRNA HOTAIR enhances ER signaling and confers tamoxifen resistance in breast cancer. Oncogene. 2016;35(21):2746–2755.
5. Cui Y, Zhang F, Zhu C, et al. Upregulated lncRNA SNHG1 contributes to progression of non-small cell lung cancer through inhibition of miR-101-3p and activation of Wnt/β-catenin signaling pathway. Oncotarget. 2017;8(11):17785–17794.
6. Liao J, Yu X, Hu X, et al. lncRNA H19 mediates BMP9-induced osteogenic differentiation of mesenchymal stem cells (MSCs) through Notch signaling. Oncotarget. 2017;8(32):53581–53601.
7. Liu T, Han Z, Li H, et al. LncRNA DLEU1 contributes to colorectal cancer progression via activation of KPNA3. Mol Cancer. 2018;17(1):118.
8. Li W, Xie P, Ruan WH. Overexpression of lncRNA UCA1 promotes osteosarcoma progression and correlates with poor prognosis. J Bone Oncol. 2016;5(2):80–85.
9. Shen H, Wang Y, Shi W, et al. LncRNA SNHG5/miR-26a/SOX2 signal axis enhances proliferation of chondrocyte in osteoarthritis. Acta Biochim Biophys Sin (Shanghai). 2018;50(2):191–198.
10. Chen Y, Du H, Bao L, et al. LncRNA PVT1 promotes ovarian cancer progression by silencing miR-214. Cancer Biol Med. 2018;15(3):238–250.
11. Huang C, Liu S, Wang H, et al. LncRNA PVT1 overexpression is a poor prognostic biomarker and regulates migration and invasion in small cell lung cancer. Am J Transl Res. 2016;8(11):5025–5034.
12. Li Y, Li S, Luo Y, et al. LncRNA PVT1 Regulates Chondrocyte Apoptosis in Osteoarthritis by Acting as a Sponge for miR-488-3p. DNA Cell Biol. 2017;36(7):571–580.
13. Lu X, Yu Y, Yin F, et al. Knockdown of PVT1 inhibits IL-1β-induced injury in chondrocytes by regulating miR-27b-3p/TRAF3 axis. Int Immunopharmacol. 2020;79:106052.
14. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–408.
15. Park G, Horie T, Fukasawa K, et al. Amelioration of the Development of Osteoarthritis by Daily Intake of β-Cryptoxanthin. Biol Pharm Bull. 2017;40(7):1116–1120.
16. Hwang HS, Kim HA. Chondrocyte Apoptosis in the Pathogenesis of Osteoarthritis. Int J Mol Sci. 2015;16(11):26035–26054. Published 2015 Oct 30.
17. Xu J, Xu Y. The lncRNA MEG3 downregulation leads to osteoarthritis progression via miR-16/SMAD7 axis. Cell Biosci. 2017;7:69. Published 2017 Dec 13.
18. Akhtar N, Miller MJ, Haqqi TM. Effect of a Herbal-Leucine mix on the IL-1β-induced cartilage degradation and inflammatory gene expression in human chondrocytes. BMC Complement Altern

Med. 2011;11:66. Published 2011 Aug 19.

19. Gao Y, Liu S, Huang J, et al. The ECM-cell interaction of cartilage extracellular matrix on chondrocytes. Biomed Res Int. 2014;2014:648459.
20. Yang H, Wu D, Li H, et al. Downregulation of microRNA-448 inhibits IL-1β-induced cartilage degradation in human chondrocytes via upregulation of matrilin-3. Cell Mol Biol Lett. 2018;23:7. Published 2018 Feb 22.
21. Zhao L, Kong H, Sun H, et al. LncRNA-PVT1 promotes pancreatic cancer cells proliferation and migration through acting as a molecular sponge to regulate miR-448. J Cell Physiol. 2018;233(5):4044–4055.
22. Zuo Y, Li Y, Zhou Z, et al. Long non-coding RNA MALAT1 promotes proliferation and invasion via targeting miR-129-5p in triple-negative breast cancer. Biomed Pharmacother. 2017;95:922–928.
23. Kim J, Yao F, Xiao Z, et al. MicroRNAs and metastasis: small RNAs play big roles. Cancer Metastasis Rev. 2018;37(1):5–15.
24. Rasheed Z, Al-Shobaili HA, Rasheed N, et al. Integrated Study of Globally Expressed microRNAs in IL-1β-stimulated Human Osteoarthritis Chondrocytes and Osteoarthritis Relevant Genes: A Microarray and Bioinformatics Analysis. Nucleosides Nucleotides Nucleic Acids. 2016;35(7):335–355.
25. Wang YX, Zhu HF, Zhang ZY, et al. MiR-384 inhibits the proliferation of colorectal cancer by targeting AKT3. Cancer Cell Int. 2018;18:124. Published 2018 Sep 3.
26. Stahl JM, Sharma A, Cheung M, et al. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer Res. 2004;64(19):7002–7010.
27. Ding L, Zhang L, Biswas S, et al. Akt3 inhibits adipogenesis and protects from diet-induced obesity via WNK1/SGK1 signaling. JCI Insight. 2017;2(22):e95687. Published 2017 Nov 16.
28. Zhang Y, Wang F, Chen G, et al. LncRNA MALAT1 promotes osteoarthritis by modulating miR-150-5p/AKT3 axis. Cell Biosci. 2019;9:54. Published 2019 Jul 1.
29. Li P, Yao Y, Ma Y, et al. MiR-150 attenuates LPS-induced acute lung injury via targeting AKT3. Int Immunopharmacol. 2019;75:105794.
30. Li R, Liu J, Li Q, et al. miR-29a suppresses growth and metastasis in papillary thyroid carcinoma by targeting AKT3. Tumour Biol. 2016;37(3):3987–3996.

Declarations

Competing interests: The authors declare no competing interests.

Figures

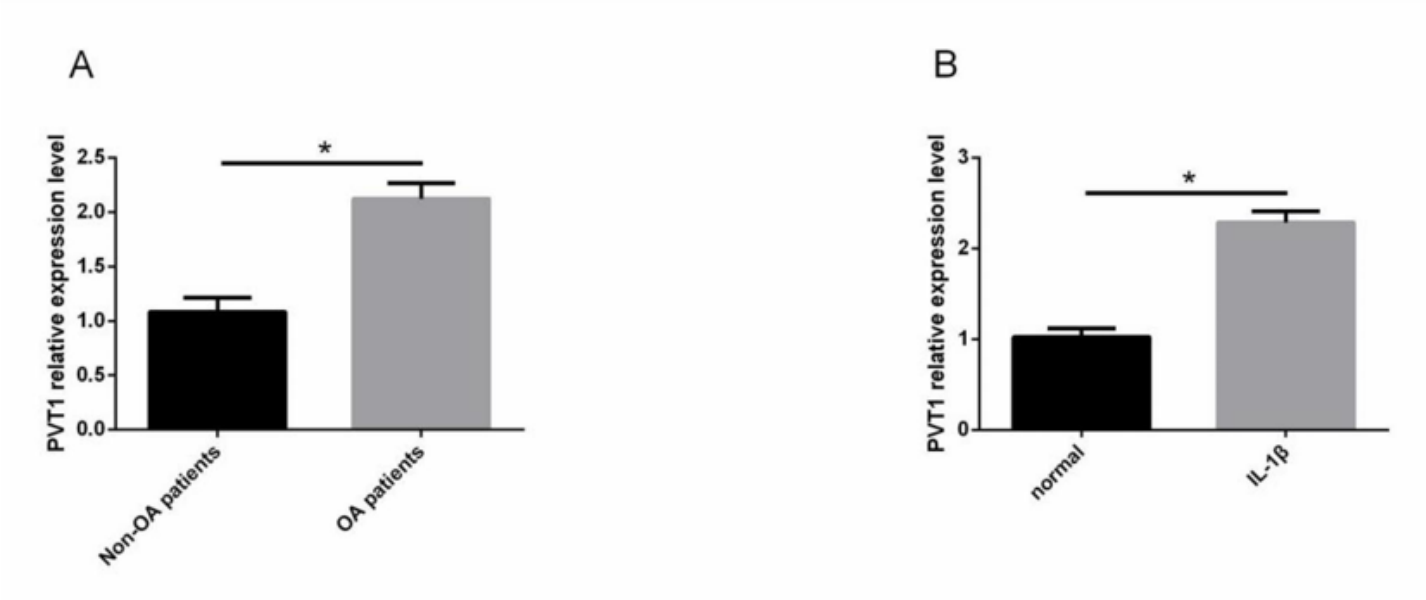

Figure 1

PVT1 is elevated in OA cartilage tissue and chondrocytes induced by IL-1β A: Detection by qRT-PCR reveals that PVT1 expression in the cartilage tissue of OA group is much higher than that of the non-OA group. B: Detection by qRT-PCR reveals that PVT1 expression is elevated in chondrocytes induced by IL-1β. Notes: Data are expressed as mean±standard devia-tion(SD) (n=24); * $p<0.05$.

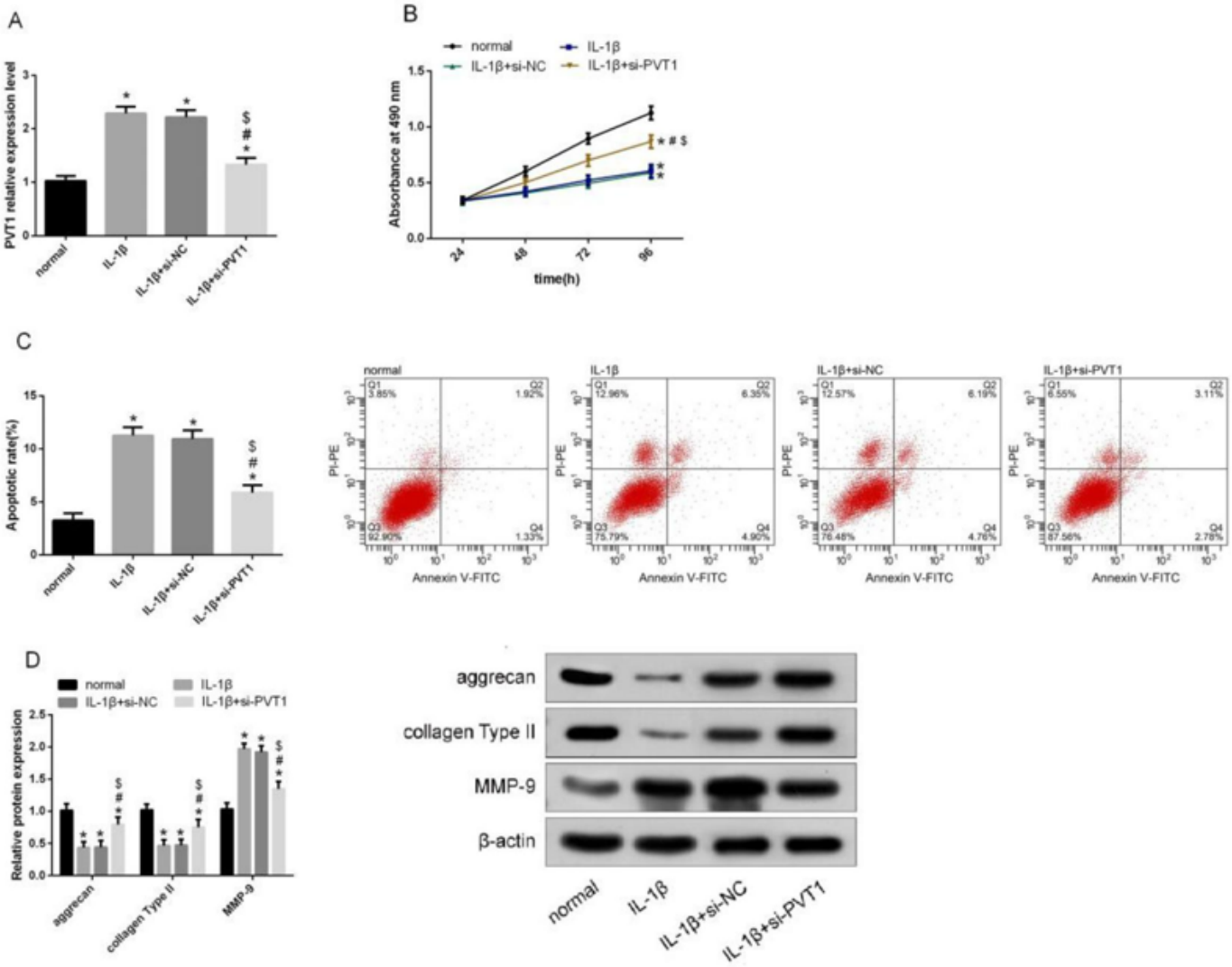

Figure 2

Effects of PVT1 on the proliferation, apoptosis and cell injury of OA cells A: Detection by qRT-PCR reveals that PVT1 expression is elevated in chondrocytes after IL-1β induction. Following the transfection of si-PVT1 into IL-1β-induced chondrocytes, PVT1 ex-pression is reduced. B: The MTT assay shows that chondrocytes induced by IL-1β have decreased proliferation ability. Following the transfection of si-PVT1 into IL-1β-induced chondrocytes, cell proliferation ability is enhanced. C: FCM shows that chondrocytes induced by IL-1β have increased cell apoptotic rates. Following the transfection of si-PVT1 into IL-1β-induced chondrocytes, cell apoptotic rate is decreased. D: WB analysis shows that chondrocytes induced by IL-1β have de-creased aggrecan and collagen Type II and increased MMP-9. Following the transfection of si-PVT1 into IL-1β-induced chondrocytes, aggrecan and collagen Type II are increased, while MMP-9 is decreased. Notes: Data are expressed as mean±standard deviation(SD)(n=24); *P < 0.05 vs normal group; #P < 0.05 vs IL-1β group; $P < 0.05 vs IL-1β+si-NC group.

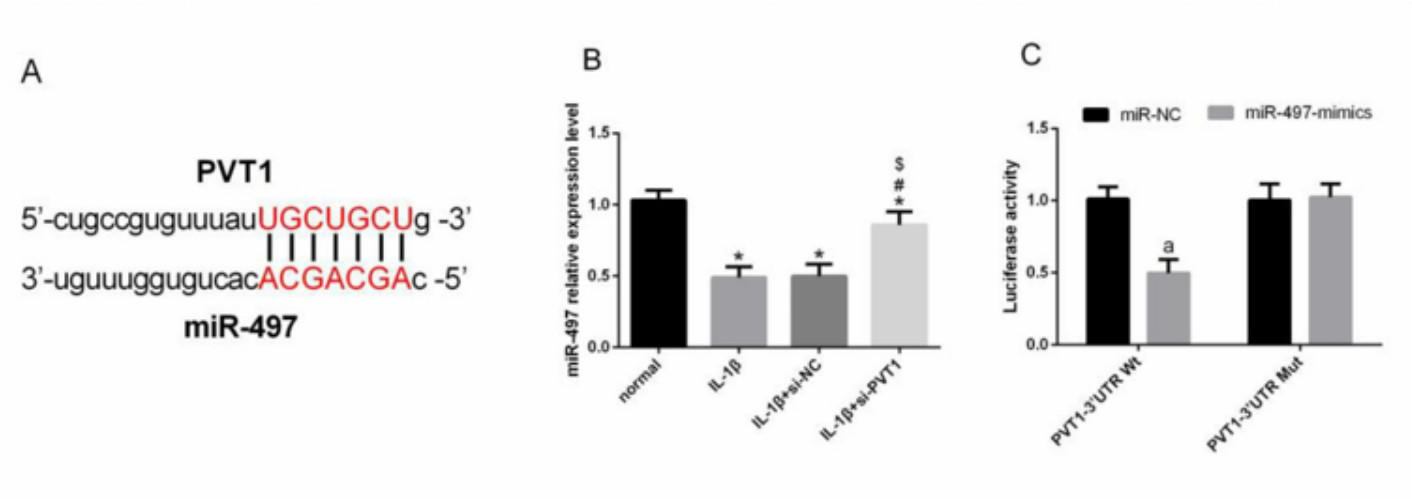

Figure 3

The interplay of miR-497 and PVT1 A: There were targeted binding sites between miR-497 and PVT1. B: Detection by qRT-PCR reveals that miR-497 expression is decreased in chondrocytes induced by IL-1β. Following the transfection of si-PVT1 into IL-1β-induced chondrocytes, miR-497 expression is reduced. C: Luciferase activity report shows that transfection with miR-497 mimics can inhibit the luciferase activity of PVT1-3 'UTR Wt without affecting the luciferase activity of PVT1-3'UTR Mut. Notes: Data are expressed as mean±standard deviation(SD) (n=24) ;*P < 0.05 vs normal group; #P < 0.05 vs IL-1β group; $P < 0.05 vs IL-1β+si-NC group; aP 0.05 VS miR-NC group.

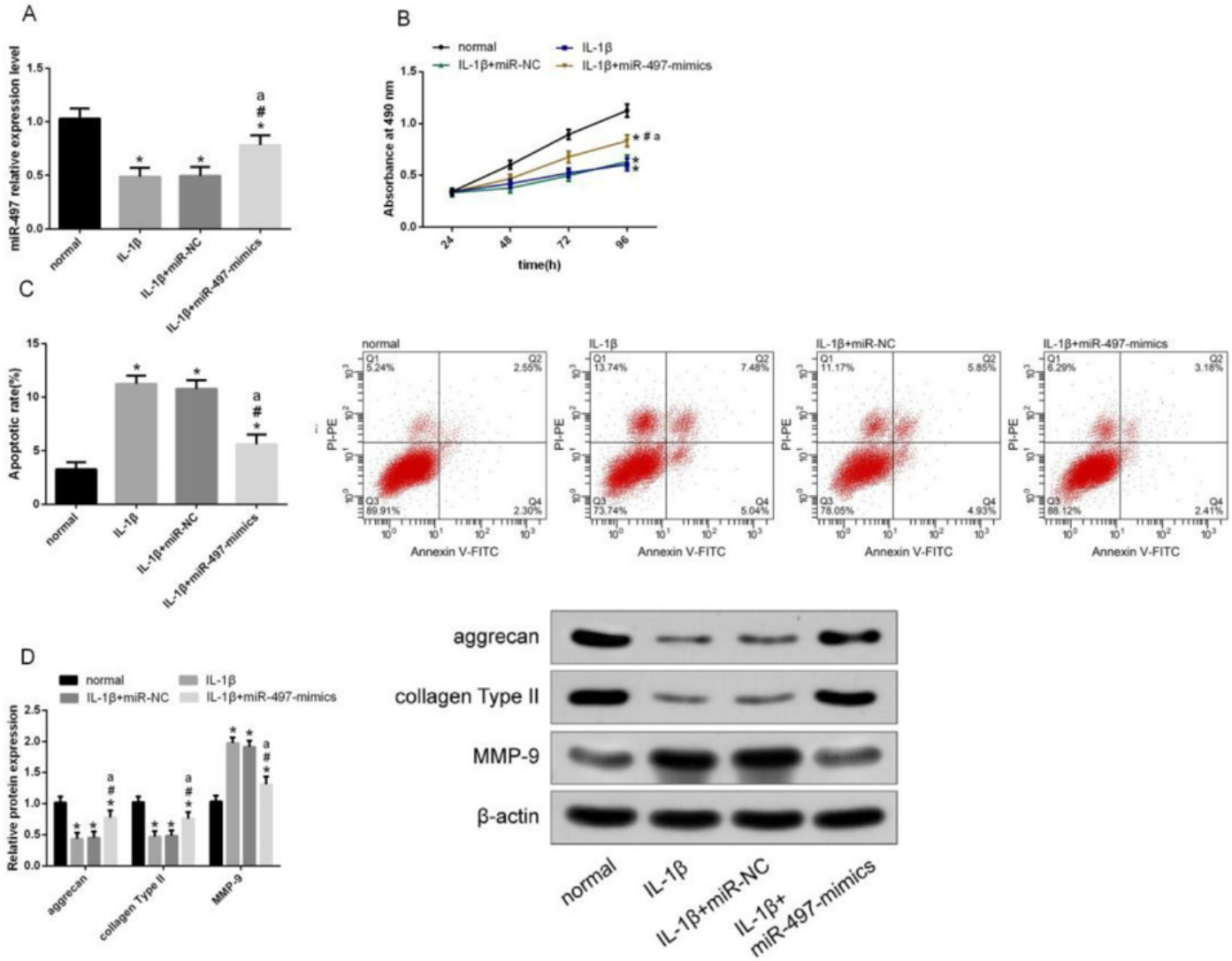

Figure 4

The function of miR-497 is the opposite of PVT1 A: Detection by qRT-PCR reveals that miR-497 expression is elevated after transfection of miR-497-mimics into IL-1β-induced chondrocytes. B: The MTT assay shows that cell proliferation ability is enhanced after transfection of miR-497 mimics into IL-1β-induced chondrocytes. C: FCM shows that the cell apoptotic rate is decreased after transfection of miR-497 mimics into IL-1β-induced chondrocytes. D: WB analysis shows that aggrecan and collagen Type II are increased while MMP-9 is decreased after the transfection of miR-497 mimics into IL-1β-induced chondrocytes. Notes: Data are expressed as mean±standard deviation(SD) (n=24); *P < 0.05 vs normal group; #P < 0.05 vs IL-1β group; $P < 0.05 vs IL-1β+si-NC group; aP□0.05 VS miR-NC group. Fig. 5 miR-497 can act through negative regulation of AKT3

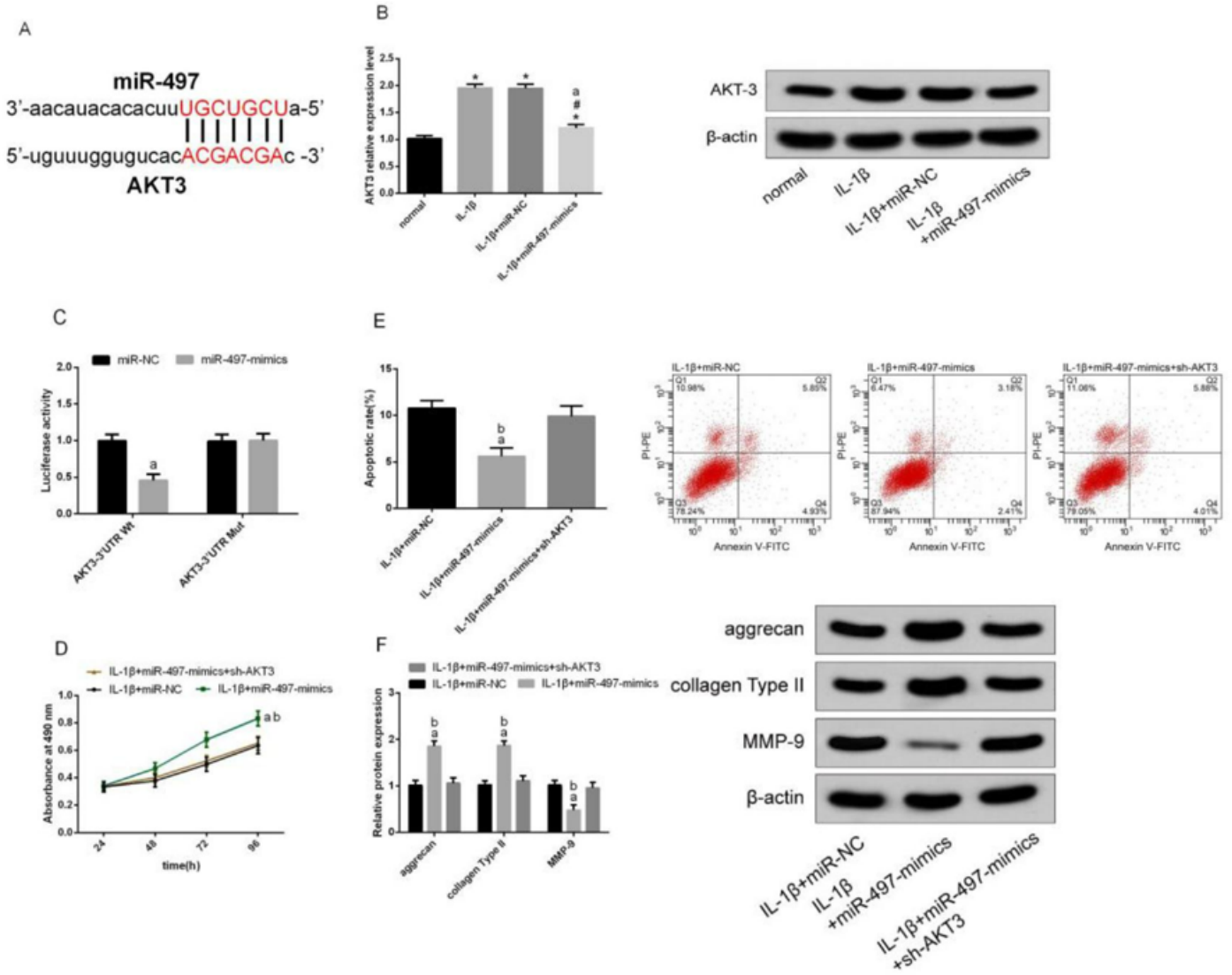

Figure 5

miR-497 can act through negative regulation of AKT3 A: DA: Binding sites between AKT3 and miR-497. B: WB analysis reveals that AKT3 expression is increased in chondrocytes induced by IL-1β. Following the transfection of miR-497 mimics into IL-1β-induced chondrocytes, AKT3 expression is reduced. C: Luciferase activity report shows that miR-497 mimics can inhibit the luciferase activity of AKT3-3' UTR Wt without causing significant changes in the luciferase activity of AKT3-3' UTR Mut. D: FCM demonstrates that cell proliferation ability is decreased in cells transfected with miR-497 mimics+sh-AKT3 compared with cells transfected with miR-497 mimics. E: Cell apoptotic rate is increased in cells transfected with miR-497 mimics+sh-AKT3 compared with cells transfected with miR-497 mimics. F: WB analysis reveals that aggrecan and collagen Type II are decreased, while MMP-9 is increased in cells transfected with miR-497 mimics+sh-AKT compared with cells transfected with miR-497 mimics. Notes: Data are expressed as mean±standard deviation(SD) (n=24) ;*P < 0.05 vs normal group; #P < 0.05 vs IL-1β group; aP□0.05 VS miR-NC group; bP□0.05 VS IL-1β+miR-497 mimics group.

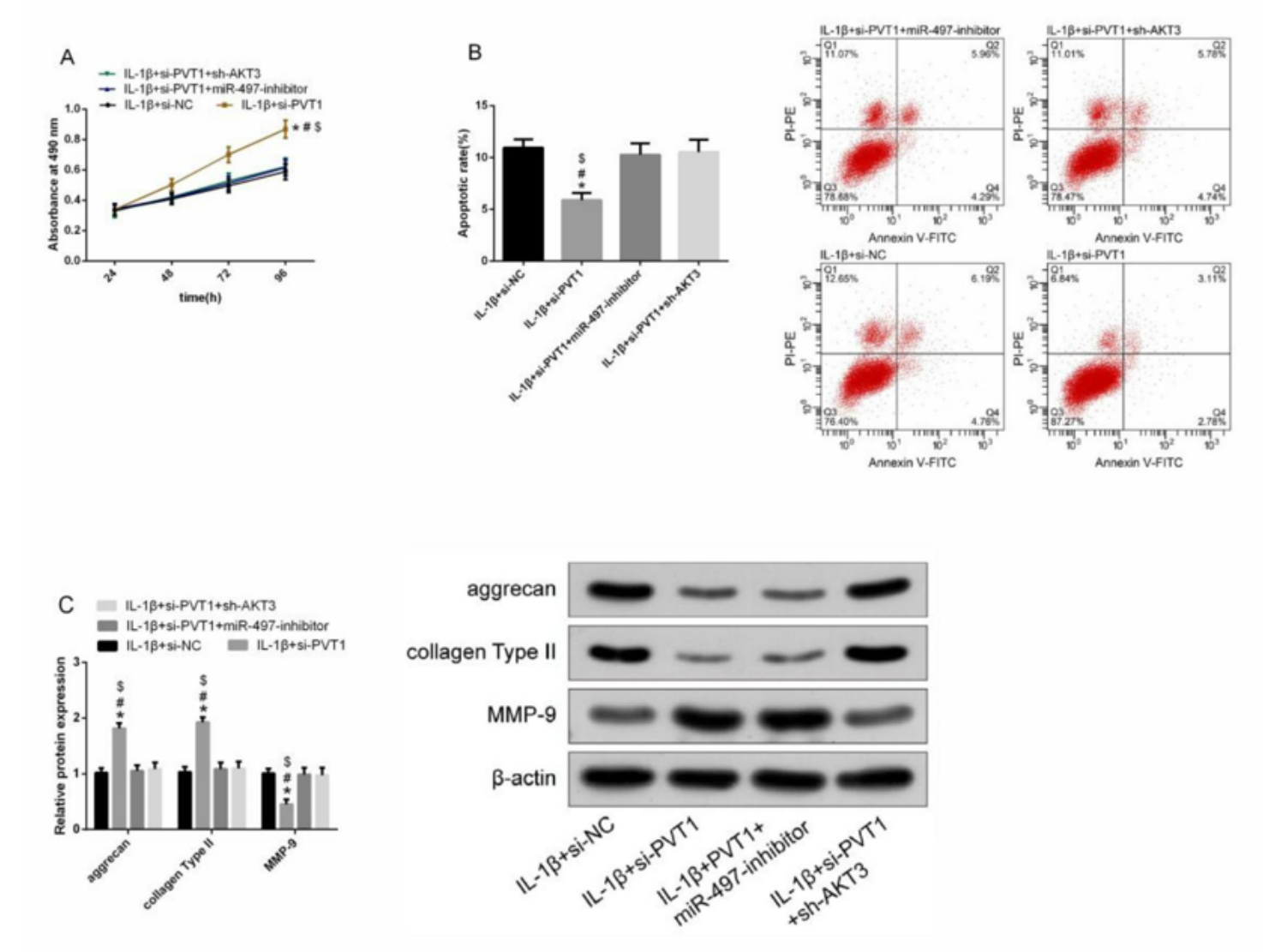

Figure 6

The regulation of chondrocytes on PVT1 depends on the regulation of the miR-497/AKT3 axis A: A: The MTT assay indicates that transfection of either miR-497-inhibitor or sh-AKT3 can reverse the effects of transfection of si-PVT1 on IL-1β-induced chondrocyte proliferation. B: FCM reveals that transfection of either miR-497 inhibitor or sh-AKT3 can reverse the effects of transfection of si-PVT1 on IL-1β-induced chondrocyte apoptosis. C: WB analysis reveals that transfection of either miR-497-inhibitor or sh-AKT3 can reverse the effects of transfection of si-PVT1 on aggrecan, collagen and MMP-9 in IL-1β-induced chondrocytes. Notes: Data are expressed as mean±standard deviation(SD) (n=24) ;*P□0.05 VS IL-1β+si-NC group□#P□0.05 VS IL-1β+si-PVT1+miR-497 inhibitor group; $P□0.05 VS IL-1β+si-PVT1+sh-AKT3 group.