LncRNA OIP5-AS1 targets miR-25-3p to accelerate intervertebral disc degeneration

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Abstract

Background

Long noncoding RNAs (lncRNAs) have been validated to exert vital roles in intervertebral disc degeneration (IDD). However, the effect of OIP5-AS1 on IDD is unknown. This research intends to explore the function of OIP5-AS1 in IDD.

Methods

RT-PCR was utilized to detect levels of OIP5-AS1, miR-25-3p, Collagen II and Aggrecan in IDD tissues and NPCs. Immunofluorescence assay measured Collagen II expression. CCK-8, EdU, and flow cytometry estimated the levels of proliferation and apoptosis. Collagen II and Aggrecan proteins were assessed via western blot. The binding affinity of OIP5-AS1 with miR-25-3p was investigated by luciferase reporter assay. Enzyme-linked immunosorbent assay (ELISA) dissected the levels of inflammatory factors such as IL-6, TNF-α, IL-10 and IL-1β.

Results

OIP5-AS1 was high expressed in IDD tissues and its expression gradually promoted with the increasing of Pfirrmann scores. The cell morphology of NPCs changed into spindle-shaped, and Collagen II expression was low. After OIP5-AS1 was silenced, cell proliferation was boosted whereas both apoptosis and extracellular matrix (ECM) degradation were restrained. In LPS-activated NPCs, OIP5-AS1 depletion also suppressed inflammation response. Further, miR-25-3p was a target of OIP5-AS1. The effects of OIP5-AS1 silence on proliferation, apoptosis and ECM degradation were reversed upon miR-25-3p downregulation. Moreover, the inhibitory impact of OIP5-AS1 knockdown on the inflammation of LPS-treated NPCs was rescued with miR-25-3p inference.

Conclusion

Totally, lncRNA OIP5-AS1 targeting miR-25-3p exerted promoting effects in IDD due to its high expression, implying the usage of OIP5-AS1/miR-25-3p as a novel regulatory axis for the molecular targets of IDD therapy.

Introduction

Intervertebral disc degeneration (IDD) is a type of multi-factorial disease that frequently evoking low back pain [1, 2]. It is attributed to internal and external factors such as senescence, genetics, autoimmune, as well as mechanical compression [3, 4], accompanied with complex aetiology [5]. In spinal surgery, some nursing methods are commonly used as assistants. For example, (1) surgical safety nursing: Prone

position is usually selected for spinal surgery. (2) Surgical environment care. (3) Nursing care of electrosurgical operation. (4) Intraoperative observation. In general, the implementation of high-quality nursing cooperation during spinal surgery can effectively improve the surgical effect of patients and reduce the possibility of complications. Further, IDD imposes a heavy burden and socioeconomic costs on patients globally [6, 7]. It is therefore of great significance to seek for the potential modulatory axis in IDD.

Compelling evidence has illustrated that during the pathogenesis of IDD, long noncoding RNAs (lncRNAs) are pivotal molecules by functioning in nucleus pulposus cells (NPCs) [8, 9]. LncRNAs are long RNA transcripts with size over 200 nucleotides (nt) and incapable of encoding proteins [10, 11]. However, the amounts of researches studying the role of lncRNAs in IDD remain rare. Among dysregulated lncRNAs, lncRNA OIP5 antisense RNA 1 (OIP5-AS1) is one functional molecule in diverse diseases. For instance, OIP5-AS1 facilitates the apoptosis of ox-LDL-treated vascular endothelial cells via GSK-3β by EZH2 recruitment [12]. Depletion of OIP5-AS1 promotes cell viability, hinders apoptosis and restricts LDH release in ox-LDL-induced HUVECs [13]. Nowadays, there is no study involving the part that OIP5-AS1 plays in IDD.

MicroRNAs (miRs) are also another subclass of noncoding RNAs (ncRNA) below 25 nt in length [14, 15]. Here, miR-25-3p is investigated for its interaction with OIP5-AS1. Reports have already revealed the promoting or inhibiting role of miR-25-3p in multiple diseases, IDD concluded. It has been delineated that miR-25-3p lowers expression of ADAM10 to hamper the formation of cardiomyocytes by P19 differentiation via inhibiting Notch signaling [16]. Besides, FOXD2-AS1 blocks miR-25-3p/Sema4C axis to boost the invasion and migration in CRC [17]. MiR-25-3p also attenuates proliferation capacity of tongue squamous cell carcinoma Tca8113 cells [18]. Moreover, miR-25-3p has been indicated to enhance NPCs proliferation and apoptosis resistance in IDD by reduction of Bim [19].

In this study, the expression pattern of OIP5-AS1 in IDD tissues was excavated. Function and downstream regulatory axis of OIP5-AS1 were further explored via CCK-8, EdU, flow cytometry, western blot and mechanism experiments. We observed that expression of OIP5-AS1 was evidently upregulated in IDD tissues and NPCs, and it exerted promoting effect through decreasing cell proliferation, as well as accelerating cell apoptosis, ECM degradation and inflammation response via targeting miR-25-3p.

Materials And Methods

Tissue samples

The Ethics Committee of the Traditional Chinese Medical Hospital of Lianyungang approved this study in patients with IDD. All participants signed the written informed consent. IDD tissues and control tissues were respectively collected from two groups of patients and next frozen in liquid nitrogen at -80°C. According to the MRI scans, IDD degree was classified utilizing the modified Pfirrmann classification.

Cell culture and treatment

Human degenerative NPCs used in this paper were extracted from NP tissues of IDD patients. The isolated NP tissues were rinsed utilizing PBS and subsequently minced into pieces, followed by digestion via 0.2% collagenase II (Crescent Chemical, Islandia, NY, USA) and 0.25% trypsin (Gibco, Carlsbad, CA, USA). Thereafter, NPCs were grown in DMEM/F12 (Gibco) with 10% FBS (Gibco), 100 U/mL penicillin as well as 100 mg/mL streptomycin under the condition of 37°C with 5% CO_2. NPCs at the third passage were used for later experiments. 1 μg/ml of LPS (cat. no. L2630; Sigma-Aldrich) was utilized to stimulate NPCs in serum-free DMEM for 1 d.

Cell transfection

Short harpin RNA targeting OIP5-AS1 (sh-OIP5-AS1; 5'-CCGGGCTCCTAGGATTCCAGTTATCCTCGAGGCAGAAGGCTGAGTTTCATTTTTTTTG-3') with the negative control sh-NC (5'-CACCGTTCTCCGAACGTGTCACGTCAAGAGATTACGTG ACACGTTCGGAGAATTTTTTG-3'), miR-25-3p mimic (Sense 5'-UCC CUG AGA CCC UAA CUU GUG A-3'; antisense 5'-ACA AGU UAG GGU CUC AGG GAU U-3') with mimic NC were obtained from GenePharma (Shanghai, China). Transfection in NPCs was conducted via Lipofectamine 2000 (Invitrogen) as per the instructions from the manufacturer.

RT-PCR

Tissues or NPCs were prepared for isolation of total RNA using TRIzol reagent (Invitrogen) as per the manufacturer's guidebook. Then reverse transcription of 1-μg RNA into cDNA was carried out with the use of the 1st Strand cDNA Synthesis Kit (TAKARA). RT-PCR was conducted on the ABI 7500 real-time PCR system (Applied Biosystems) with the SYBR-Green PCR Master Mix (Roche) following the introductions. The parameters for PCR were: 10-min pre-treatment at 95°C; 40 cycles at 95˚C for 15 sec; at 60°C for 1 min; and at 72˚C for 5 min. The $2^{-\Delta\Delta Ct}$ method was applied for calculation of gene expression that was normalized to GAPDH or U6. Utilized primers were as follows: OIP5-AS1 sense, 5'-GGTCGTGAAACACCGTCG-3' and antisense, 5'-GTGGGGCATCCAGGGT-3'; Collagen II sense, 5'-CTGGTGATGATGGTGAAG-3' and antisense, 5'-CCTGGATAACCTCTGTGA-3'; Aggrecan sense, 5'-CAGATGGCACCCTCCGATAC-3' and antisense, 5'-GACACACCTCGGAAGCAGAA-3'; GAPDH sense, 5'-AATCCCATCACCATCTTCCAG-3' and antisense, 5'-TGATGACCCTTTTGGCTCCC-3'; miR-25-3p sense, 5'-CATTGCACTTGTCTCGGTCTGA-3' and antisense 5'-GCTGTCAACGATACGCTACGTAACG-3'; U6 sense, 5'-CTCGCTTCGGCAGCACA-3' and antisense, 5'-AACGCTTCACGAATTTGCGT-3'.

Immunofluorescence

NPCs were fastened utilizing 4% paraformaldehyde for just 0.5 h, cultured with 0.1% Triton X-100 for another 15 min, and subsequently blocked via applying 2% bovine serum albumin (Sigma) for extra 1 h. After that, NPCs were cultivated with primary antibody against collagen II (1:200; Santa Cruz Biotechnology) for whole night at 4°C, followed by exposure to secondary antibodies labelled with Alexa Fluor® 594 (1:100; Life Technologies) at indoor temperature for 1 h. After thrice washes by PBS, NPCs were stained via DAPI. Photos were observed by laser confocal microscopy (OLYMPUS).

CCK-8 experiment

To assess cell viability, a commercial Cell Counting Kit-8 (CCK-8; Dojindo) was utilized. NPCs (5×10^3 cells/well) were placed in 96-pore dishes and 10-μl CCK-8 solution was supplemented for extra culture of 2 h. Absorbance in each hole was estimated at 450 nm.

EdU assay

96-well plates (1×10^4 cells/well) were prepared for culture of NPCs. For this assay, Cell-Light^{TM} EDU Apollo 488 kit (Ruibio, China) was employed. Cells were dyed with Hoechst 33342. To assess cell proliferation rate, the ratio of the EdU positive cells/Hoechest staining cells selected randomly at three fields was calculated. Fluorescence microscope (Leica Microsystems) was utilized for observation.

Flow cytometry

Apoptosis ability was evaluated via Annexin V-FITC-propidium iodide (PI) apoptosis detection reagent (BD Biosciences) as the manufacturer guided. Cells were first rinsed thrice utilizing PBS, digested using trypsin (1 ml) and next adjusted to the density of 1×10^5 cells/100 μl for resuspension in 1X Annexin binding buffer. Next, cells were collected and dyed via applying the above reagent for 15 min. The apoptosis rate was quantified with flow cytometry 10.0 (FlowJo, FACS CaliburTM, BD Biosciences).

Western blot

To extract total protein, an RIPA lysis buffer was employed. Also, a BCA Protein Assay Kit (Thermo Scientific) was utilized for protein concentration. Equivalent protein (20 μg) was separated by employing 10% SDS-PAGE, which was transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore). Blocking of mixture was implemented by use of 5% fat-free milk for extra 1 h at room temperature. Next, primary antibodies against Collagen II, Aggrecan and GAPDH (1:1,000; Abcam) were adopted at 4 °C for one night. Secondary culture was implemented with HRP-labelled goat anti-rabbit antibody (Boster, Wuhan, China). ECL Plus reagent (Millipore, USA) was adopted for visualization of signals. The intensity of bands was quantified via Image Lab 3.0 software (Bio-Rad, CA, USA).

ELISA

The contents of IL-6, TNF-α, IL-10, and IL-1β were estimated through relative ELISA kits (eBioscience) following the producer's instructions. Under the wavelength at 450 nm, all samples were assessed with a microplate reader (SpectraMax M5, Molecular Devices). Next, a standard curve was plotted with the help of computer software based on the absorbance value.

Luciferase reporter assay

The wild- and mutant-type OIP5-AS1 with or without putative binding sites for miR-25-3p (OIP5-AS1-WT/MUT) were constructed into pmiRGLO (Invitrogen). After that, NPCs were co-transfected with miR-25-

3p or miR-NC with OIP5-AS1-WT or OIP5-AS1-MUT, the procedures of which were implemented utilizing Lipofectamine 2000 (Invitrogen). 48 h post transfection, the luciferase activity in each group was analyzed using a dual-luciferase reporter system (Promega). Renilla luciferase activity acted as the normalization.

Statistical analysis

The gained data of triplicated experiments were dissected by SPSS v.18.0 software (SPSS Inc., Chicago, IL, USA) and represented as the mean ± standard deviation (SD). Comparisons between or among groups were analyzed via Student's t-test or one-way ANOVA. The statistics were significant upon $P < 0.05$.

Results

OIP5-AS1 was up-regulated in IDD and its silence accelerated cell proliferation

Firstly, expression of lncRNA OIP5-AS1 in tissues of IDD patients was assessed. RT-PCR delineated that the positive correlations between OIP5-AS1 levels and the patients' Pfirrmann scores from □ to □ was affirmed, hinting that high expression of OIP5-AS1 suggested more severe degree of IDD (Figure 1A). As presented in Figure 1B, NPCs isolated from tissues were star-shaped, polygonal or spindle-shaped, similar to chondroid cells. Besides, immunofluorescence assay illustrated that Collagen II expression was expressed at low level in NPCs (Figure 1C). We further explored the function of OIP5-AS1 in IDD. RT-PCR was utilized to analyze the level of OIP5-AS1 in NPCs transfected with sh-OIP5-AS1. OIP5-AS1 was dramatically lowered after sh-OIP5-AS1 treatment (Figure 1D). CCK-8 experiment proved that cell viability of NPCs was distinctly promoted by OIP5-AS1 silence (Figure 1E). Also, boosted cell proliferation by OIP5-AS1 knockdown was observed in NPCs through EdU assay (Figure 1F). It was clear that OIP5-AS1 was heightened in IDD and silence of OIP5-AS1 promoted cell proliferation.

OIP5-AS1 silence decreased cell apoptosis and ECM degradation

Then, the effect of OIP5-AS1 on cell apoptosis was assessed. Flow cytometry analysis revealed that inhibition of OIP5-AS1 remarkably hampered cell apoptosis (Figure 2A). Besides, RT-PCR and western blotting were performed to investigate the changes of Collagen II and Aggrecan, which presented that mRNA and protein expression of Collagen II and Aggrecan was obviously increased under OIP5-AS1 silencing (Figure 2B-C). Here, cell apoptosis and ECM degradation were inhibited when OIP5-AS1 was silenced.

Decrease of OIP5-AS1 declined inflammation response in LPS-induced NPCs

Next, we investigated the role of OIP5-AS1 in LPS-induced NPCs. RT-PCR demonstrated that LPS induced the expression of OIP5-AS1, which was further down-regulated by transfection of sh-OIP5-AS1 (Figure 3A). As measured by ELISA, the levels of IL-6, TNF-α and IL-1β were significantly elevated but the level of IL-10 was significantly lowered after LPS stimulation. However, OIP5-AS1 knockdown markedly reversed

the up-regulation of IL-6, TNF-α and IL-1β levels and the depletion of IL-10 level (Figure 3B). Obviously, the inflammation was suppressed with OIP5-AS1 down-regulation.

MiR-25-3p was targeted by OIP5-AS1 and low in IDD

It was predicted that OIP5-AS1 bound with miR-25-3p. The binding sites were shown in Figure 4A. In luciferase reporter assay, it was validated that the luciferase activity of OIP5-AS1-WT was overtly decreased when miR-25-3p was overexpressed. In contrast, that of OIP5-AS1-MUT had no changes in response to miR-25-3p elevation (Figure 4B). The expression of miR-25-3p was in negative correlation with Pfirrmann scores (Figure 4C). Taken together, OIP5-AS1 targeted miR-25-3p that was lowly expressed in IDD.

Reduction of miR-25-3p rescued the effects of OIP5-AS1 inhibition on proliferation, apoptosis and ECM degradation

To probe whether OIP5-AS1/miR-25-3p axis functioned in IDD, we conducted rescue experiments. As affirmed through RT-PCR, miR-25-3p silence had no effect on OIP5-AS1 expression. Whereas, OIP5-AS1 downregulation significantly promoted miR-25-3p expression, which was distinctly reduced with treatment of miR-25-3p inhibitor (Figure 5A). CCK-8 results indicated that decrease of miR-25-3p distinctly repressed cell viability. Moreover, the accelerated cell viability due to OIP5-AS1 depletion was hindered by miR-25-3p inhibition (Figure 5B). Flow cytometry analysis elucidated that cell apoptosis was markedly activated by miR-25-3p repression but restricted by OIP5-AS1 knockdown, and the obstruction was abolished with miR-25-3p inference (Figure 5C). The mRNA and protein levels of Collagen II and Aggrecan was decreased under miR-25-3p suppression but promoted under OIP5-AS1 inference, which was neutralized with co-treatments of sh-OIP5-AS1 and miR-25-3p inhibitor (Figure 5D, E). In summary, the promotion of proliferation and the inhibition of apoptosis and ECM degradation caused by reduction of OIP5-AS1 were abrogated with silencing of miR-25-3p.

Reduction of miR-25-3p rescued the effects of OIP5-AS1 inhibition on the inflammation

Also, in LPS-treated NPCs, the regulatory mechanism of OIP5-AS1/miR-25-3p axis was explored. Reduction of miR-25-3p improved the levels of IL-6, TNF-α and IL-1β whereas reduced the IL-10 level in LPS-treated NPCs. And the decreases of IL-6, TNF-α and IL-1β and the increase of IL-10 attributed to inference of OIP5-AS1 were significantly recovered when miR-25-3p was lessened (Figure 6). Collectively, the inhibition of inflammation response caused by reduction of OIP5-AS1 was abrogated with silencing of miR-25-3p.

Discussion

Experimental data unmasked that OIP5-AS1 was high expressed in IDD tissues. After OIP5-AS1 expression was knocked down, proliferation was enhanced, apoptosis as well as ECM degradation were impeded, and inflammation response was reduced. MiR-25-3p was the target of OIP5-AS1. Thereafter, the

binding between OIP5-AS1 and miR-25-3p was attested. Expression of miR-25-3p in IDD tissues was also dramatically down-regulated. OIP5-AS1 was proved to target and reduce miR-25-3p to hinder proliferation, boost apoptosis and ECM degradation, and evoke inflammation response.

Through searching on NCBI website, we discovered that lncRNA OIP5-AS1 is a promotive molecule in different diseases. During the process of atherosclerosis, lncRNA OIP5-AS1 targets miR-26a-5p to modulate AKT/NF-κB pathway and exerts promotion effect [20]. LncRNA OIP5-AS1 indicates dismal prognosis and mediates cell proliferation in bladder cancer through OIP5 [21]. Additionally, OIP5-AS1 decrement boosts cell viability, suppresses apoptosis and inhibits LDH release in HUVECs treated by ox-LDL [13]. We firstly explored the level of OIP5-AS1 in IDD tissues. Compared with control tissues, level of OIP5-AS1 was obviously increased in IDD tissues. Furthermore, with the development of IDD, OIP5-AS1 level presented increased trend. Thus, it was reasonable to suppose that OIP5-AS1 played a vital role in IDD.

The etiology of IDD composes of the reduction of NPCs due to pathological changes such as activated cell apoptosis and provoked ECM degradation, thus triggering degeneration [22, 23]. Proteoglycans (primarily aggrecan) and Collagen II are two main effectors of ECM, the decreases of which can promote the degradation of ECM [24]. In the current paper, it was affirmed that under OIP5-AS1 depletion, promoted cell proliferation, inactivated cell apoptosis and inhibited ECM degradation were observed. Besides, increased expression of inflammatory cytokines also contributes to IDD degeneration [25, 26]. The impacts of OIP5-AS1 on inflammatory factors were investigated. In LPS-treated NPCs, the elevated levels of inflammatory factors were markedly decreased with the inference of OIP5-AS1. The functional role of OIP5-AS1 in IDD was firstly unveiled by the current work. Taken together, OIP5-AS1 inference played a suppressive role in IDD.

Multiple studies have demonstrated the mechanism of lncRNAs with miRNAs in IDD. For examples, lncRNA HCG18 potentiates IDD via sponge of miR-146a-5p and regulation of TRAF6 [27]; LINC00641 modulates autophagy and IDD by interaction with miR-153-3p under stress from nutrition deprivation [28]; HOTAIR acts as a miR-34a-5p sponge to inactivate apoptosis of NPCs via NOTCH1 signaling [29]. In this research, we utilized bioinformatic tool to predict the potential genes interplaying with OIP5-AS1. Interestingly, miR-25-3p, assumed to be targeted by OIP5-AS1, has been reported as a suppressor for IDD [19]. Previous researches have also demonstrated the suppressive role of miR-25-3p in colorectal cancer [17] and tongue squamous cell carcinoma [18]. Therefore, it was speculated that OIP5-AS1 bound to miR-25-3p and accelerated the progression of IDD.

Next, mechanism experiments were conducted. Luciferase reporter assays verified the binding of OIP5-AS1 to miR-25-3p in IDD. Level of miR-25-3p in IDD tissues was distinctly decreased compared with control tissues. Also, with the increasing degree of IDD, level of miR-25-3p gradually reduced. Additionally, elevated expression of miR-25-3p was observed upon repression of OIP5-AS1, hinting the inhibitory effect of OIP5-AS1 on miR-25-3p. Further, the function of OIP5-AS1/miR-25-3p was explored via reducing the expression of miR-25-3p in the presence of OIP5-AS1 inhibition. The results demonstrated that depletion

of miR-25-3p neutralized the effects of OIP5-AS1 decrease on proliferation, apoptosis and ECM degradation. Moreover, miR-25-3p reduction rescued the effects of OIP5-AS1 decrement on inflammation cytokines. These findings affirmed that OIP5-AS1 repressed miR-25-3p expression to suppress proliferation, as well as facilitate apoptosis, ECM degradation and inflammation in IDD.

In conclusion, we indicated the high expression of OIP5-AS1 in IDD, and disclosed the stimulative impact of OIP5-AS1 on proliferation and its suppressive impacts on apoptosis, ECM degradation and inflammation. MiR-25-3p was verified to be the target of OIP5-AS1 and its depletion could reverse the effects of OIP5-AS1 on the above pathological changes. The present work provides evidence that OIP5-AS1 targets miR-25-3p to facilitate IDD, which is possibly beneficial for IDD therapy.

Abbreviations

Long noncoding RNAs: lncRNAs), intervertebral disc degeneration: IDD, Enzyme-linked immunosorbent assay: ELISA, extracellular matrix: ECM, nucleus pulposus cells: NPCs, Cell Counting Kit-8: CCK-8.

Declarations

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

Zhaoping Che participated in the design of the study. Jie Xu drafted the manuscript. Zongyu Zhang carried out the experiments and performed the statistical analysis. All authors read and approved the final manuscript.

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Figures

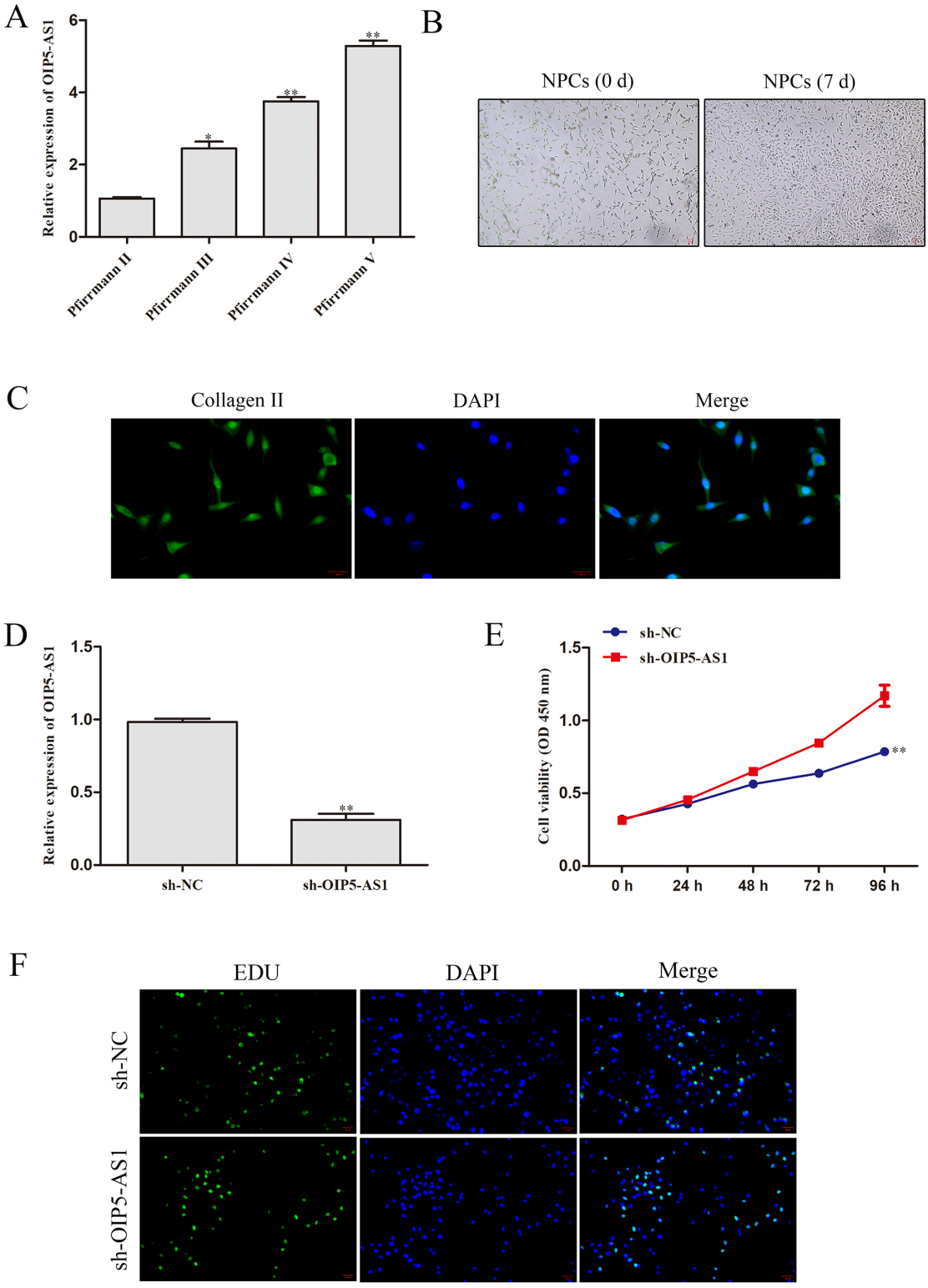

Figure 1

OIP5-AS1 was up-regulated in IDD and its silence accelerated cell proliferation. (A) RT-PCR analysis of OIP5-AS1 in groups of different Pfirrmann scores. *P < 0.05, **P < 0.01 vs. Pfirrmann. (B) Cell morphology of NPCs isolated from IDD tissues. (C) Immunofluorescence for Collagen II expression in NPCs was conducted. (D) Level of OIP5-AS1 in NPCs was estimated via RT-PCR experiment. **P < 0.01

vs. sh-NC. (E-F) CCK-8 and EdU experiments for analyzing cell proliferation ability of NPCs treated with sh-NC or sh-OIP5-AS1. ** P < 0.01 vs. sh-NC.

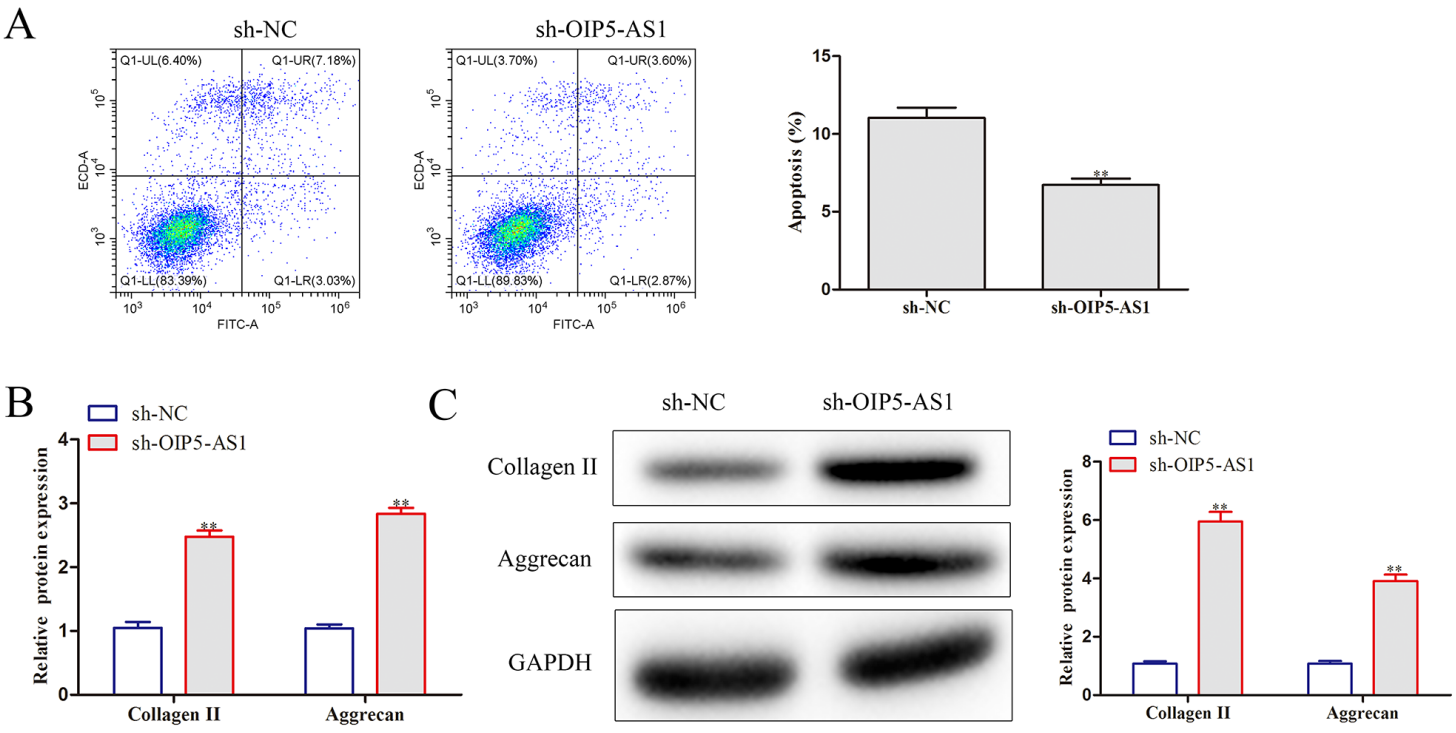

Figure 2

OIP5-AS1 silence decreased cell apoptosis and ECM degradation. (A) The apoptotic levels of NPCs after OIP5-AS1 silence were explored through flow cytometry. (B-C) RT-PCR and western blot were conducted to assess mRNA and protein expression of Collagen II and Aggrecan in OIP5-AS1-silenced NPCs. **P < 0.01 vs. sh-NC.

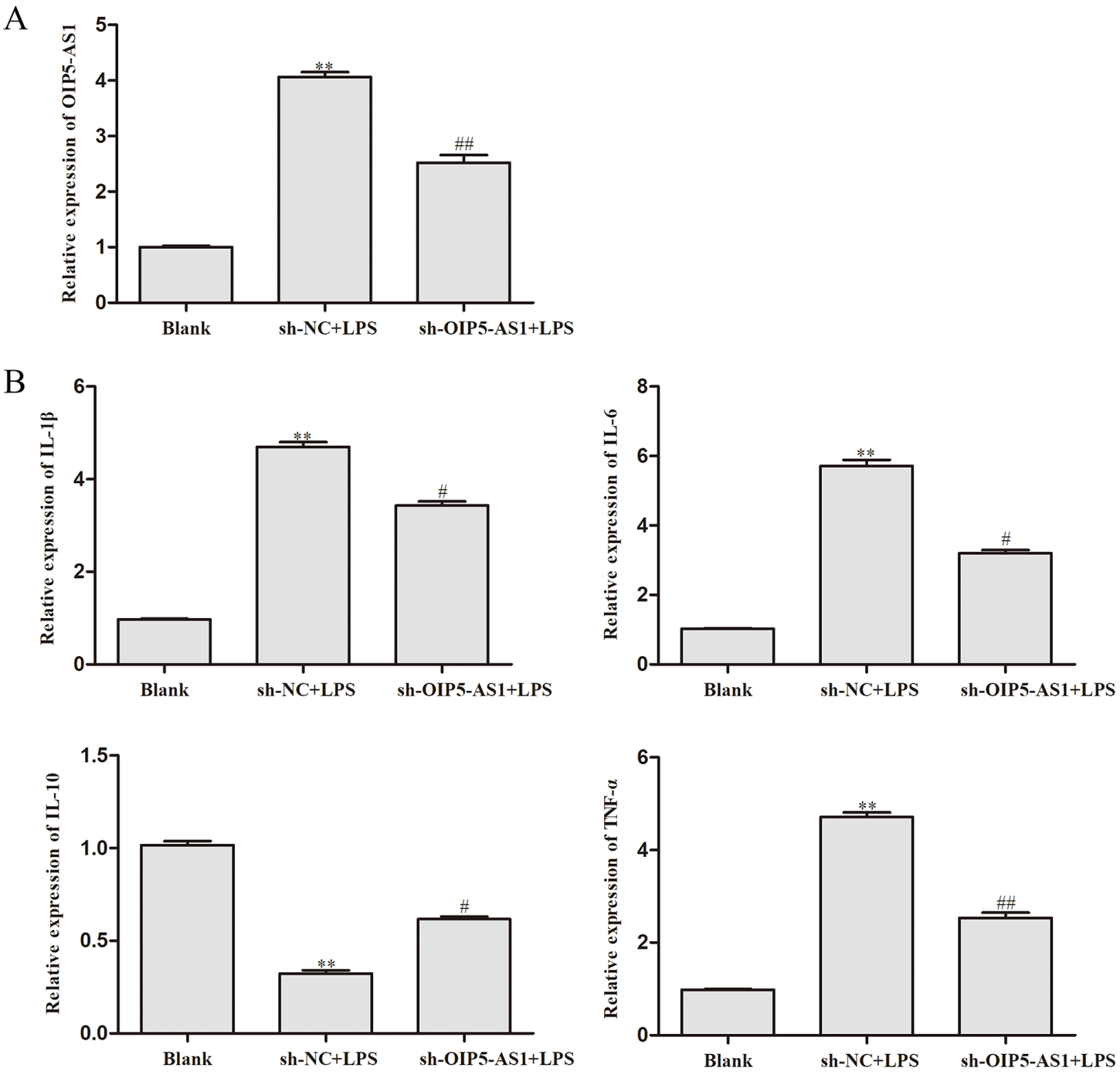

Figure 3

Decrease of OIP5-AS1 declined inflammation response in LPS-induced NPCs. (A) Levels of OIP5-AS1 in NPCs of Blank, sh-NC+LPS and sh-OIP5-AS1+LPS groups were detected through RT-PCR. (B) Levels of IL-6, TNF-α, IL-10 and IL-1β were explored via ELISA. **P < 0.01 vs. Blank, #P < 0.05, ##P < 0.01 vs. sh-NC+LPS.

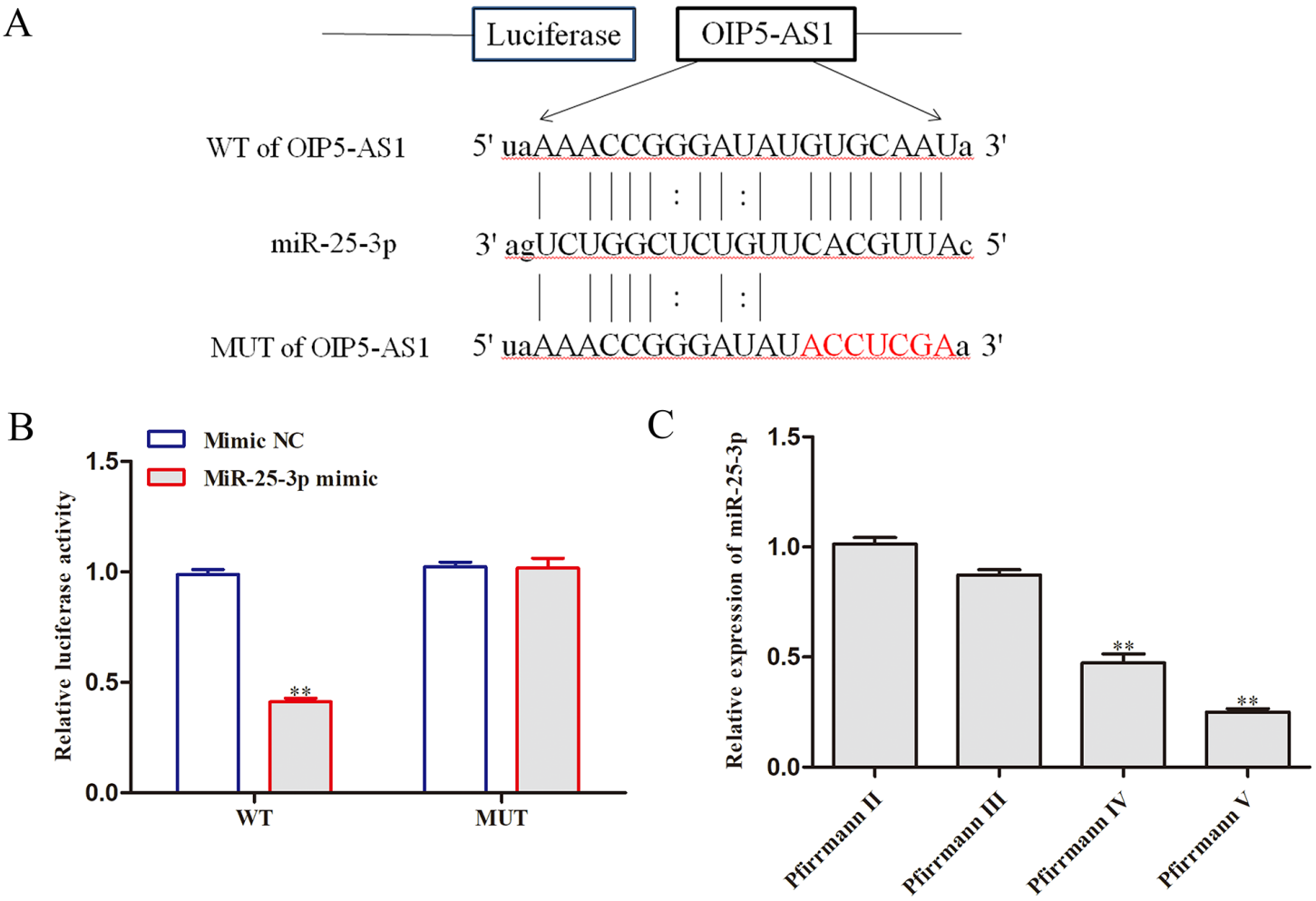

Figure 4

MiR-25-3p was targeted by OIP5-AS1 and it was low in IDD. (A) Binding sites between OIP5-AS1 and miR-25-3p as predicted by bioinformatic tools. (B) Luciferase reporter assay was applied to determine the interaction of OIP5-AS1 with miR-25-3p. **P < 0.01 vs. Mimic NC. (C) RT-PCR was employed to evaluate the level of miR-25-3p in tissues with various Pfirrmann scores. **P < 0.01 vs. Pfirrmann Ⅱ.

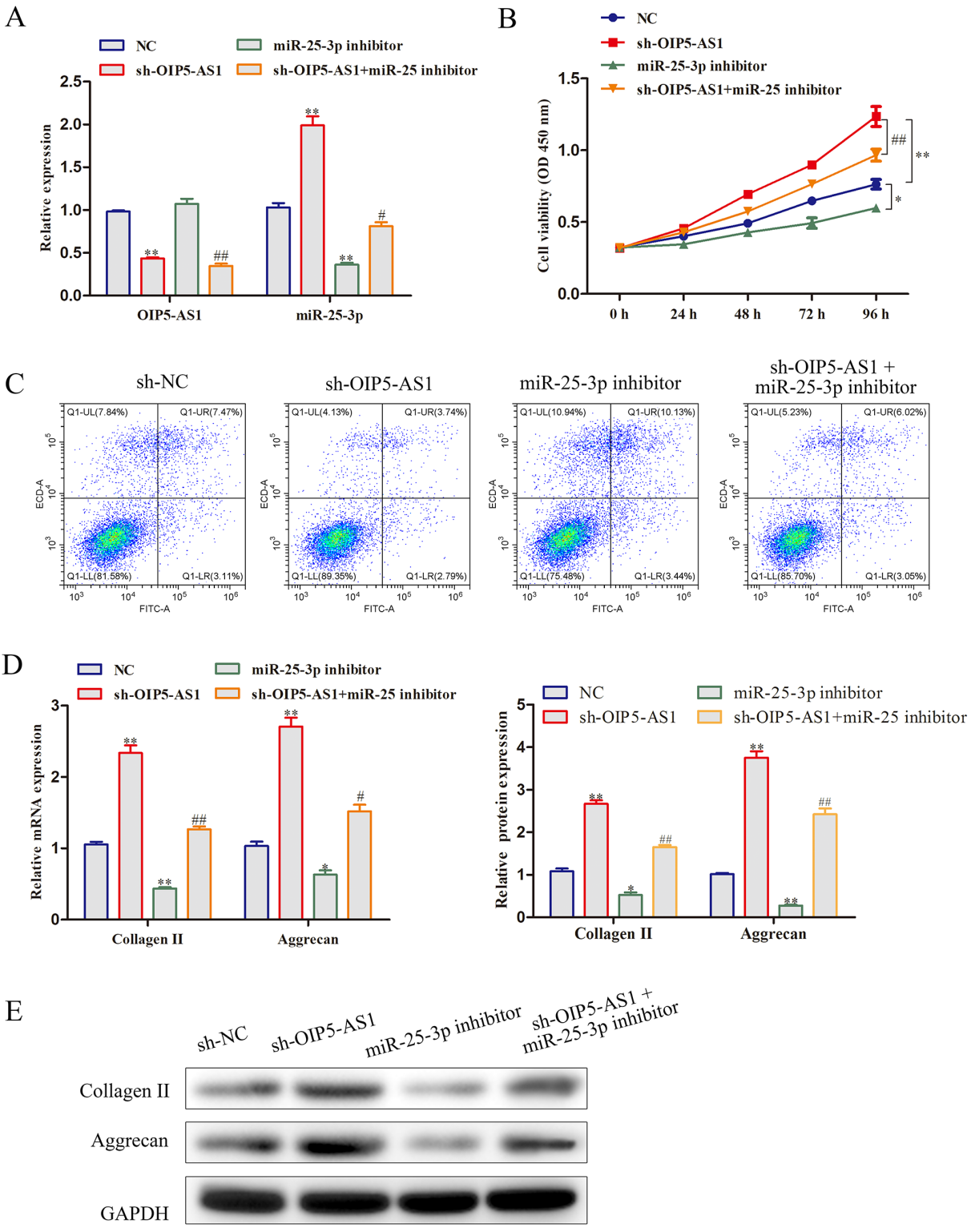

Figure 5

Reduction of miR-25-3p rescued the effects of OIP5-AS1 inhibition on proliferation, apoptosis and ECM degradation. (A) Levels of OIP5-AS1 and miR-25-3p under different transfections were explored through RT-PCR. (B) CCK-8 detection of cell proliferation of NPCs. (C) Flow cytometry test of apoptotic levels in NPCs. (D) RT-PCR and western blotting for analyzing Collagen II and Aggrecan expression in mRNA and protein levels in NPCs. *P < 0.05, **P < 0.01 vs. NC, #P < 0.05 and ##P < 0.01 vs. sh-OIP5-AS1.

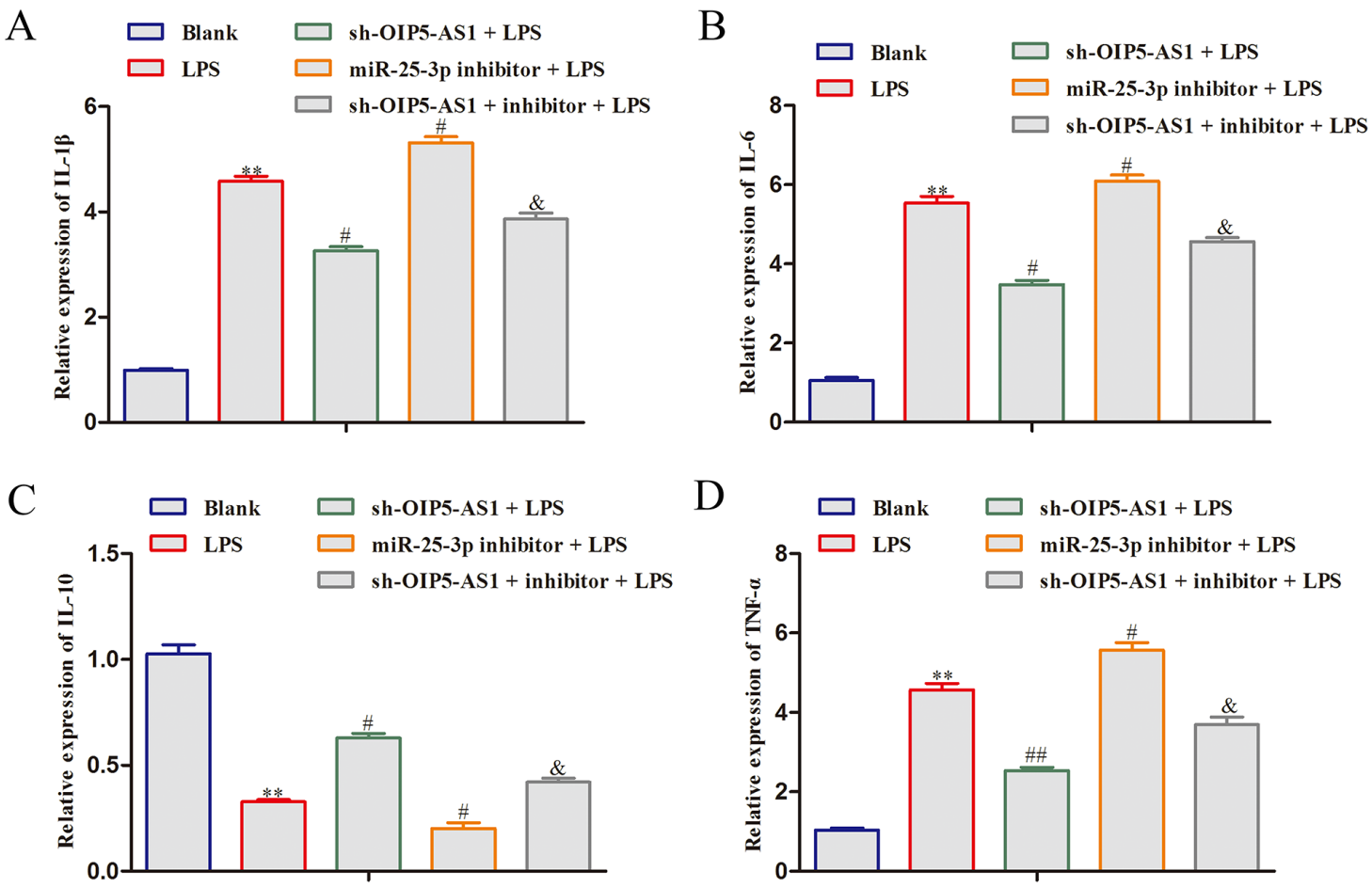

Figure 6

Reduction of miR-25-3p rescued the effects of OIP5-AS1 inhibition on the inflammation. ELISA for contents of IL-6, TNF-α, IL-10 and IL-1β in LPS-induced NPCs. **P < 0.01 vs. Blank, #P < 0.05 and ##P < 0.01 vs. LPS, &P < 0.05 vs. LPS+sh-OIP5-AS1.