Regenerative Therapy 29 (2025) 51–59

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

DDIT4 participates in high glucose-induced fibroblast-like synoviocytes overactivation and cartilage injury by regulating glycolysis

Shuo Qiang, Cheng Cheng, Yonghui Dong, Chao Tang, Jia Zheng, Yunke Liu*

Department of Orthopedics, Henan Provincial People's Hospital, Zhengzhou University People's Hospital, Zhengzhou, Henan, 450003, China

ARTICLE INFO

Article history:
Received 14 November 2024
Received in revised form
16 February 2025
Accepted 27 February 2025

Keywords:
DDIT4
Diabetic osteoarthritis
Fibroblast-like synoviocytes
Cartilage injury
Glycolysis

ABSTRACT

Objective: More and more evidence show that diabetes is closely related to osteoarthritis (OA). However, the role and mechanism of DNA damage-inducible transcript 4 protein (DDIT4) in diabetic OA (DOA) have not been clarified.

Methods: We collected OA patients and non-OA subjects who underwent total knee replacement surgery, and analyzed the DDIT4 expression in synovial samples using RT-qPCR. The cell viability of fibroblast-like synoviocytes (FLSs) was measured by CCK-8 assay. Annexin V-FITC/PI double staining was used to detect the cell apoptosis. Scratch and Transwell assays were used to determine cell migration and invasion, respectively.

Results: The levels of cellular inflammatory factors (IL-1β, IL-6 and TNF-α), oxidative stress and glycolysis related indicators were detected by using kits. Western blot was used to determine the expression of DDIT4, Aggrecan, COL3A1, MMP3, MMP13, HK2, PFKP and PKM2 in FLSs or ATDC5 cells. The results showed that the expression level of DDIT4 was significantly reduced in the synovial samples of OA patients and primary FLSs. Functional studies showed that DDIT4 overexpression inhibited the overactivation, migration, and invasion of FLSs, as well as alleviated chondrocyte injury co-cultured with FLSs. Importantly, the expression of DDIT4 was down-regulated in patients with DOA and closely related to DOA. Further research found that high glucose (HG) promoted excessive activation, migration, and invasion of FLSs, and exacerbated the followed chondrocyte injury. Overexpression of DDIT4 alleviated HG-induced abnormal function of FLSs and injury to chondrocytes. Importantly, DDIT4 inhibited lactate synthesis, glucose uptake, LDH activity, extracellular acidification rate, oxygen consumption rate, and expression levels of glycolysis related protein (HK2, PFKP, PKM2) in HG-induced FLSs. And the glycolysis inhibitors (Cyto-B and 3BrPA) alleviated the injury of ATDC5 chondrocytes co-cultured with FLSs.

Conclusions: DDIT4 participates in HG-induced FLSs overactivation and inflammation response, as well as chondrocyte injury and OA progression by regulating glycolysis processes.

1. Introduction

Osteoarthritis (OA) is the most common joint disease characterized by cartilage injury that affects the entire joint tissue, ultimately leading to cartilage degeneration, fibrosis, rupture, and defect [1]. Fibroblast-like synoviocytes (FLSs) are the main effector of OA synovial inflammation, with characteristics of abnormal proliferation, migration, adhesion, invasion, and secretion, playing an important role in the pathology of OA [2,3]. As the main cell population in synovial tissue, FLSs are believed to participate in OA progression by secreting inflammatory factors, including interleukins (IL), tumor necrosis factors (TNFs), matrix metalloproteinases (MMPs), ADAM metallopeptidases and platelet reactive protein type (ADAMTS) proteases, as well as components that regulate the ECM of chondrocytes. These components will affect the balance of chondrocyte physiological structure [4]. Therefore, understanding the action mechanism of FLSs in the process of OA will provide a help to explore the pathological background and treatment method for OA.

* Corresponding author. No.7, Weiwu Road, Jinshui District, Zhengzhou, Henan, 450003, China.
E-mail addresses: qiangshuo@zzu.edu.cn (S. Qiang), yunkelyk@126.com (Y. Liu).
Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2025.02.017

Pain is the most common and treatable symptom. The severity of pain may be affected by many factors, such as age, gender, obesity and other complications, such as diabetes [5]. Recent evidence further suggests that diabetes is closely related to the severity of pain in patients with knee OA [6]. According to the research report, the existence of diabetes may promote joint injury by two ways: chronic hyperglycemia leads to increased oxidative stress, excessive production of pro-inflammatory cytokines and advanced glycation end products in joints; insulin resistance has negative effects on local and systemic joints by promoting inflammation [7]. In addition, the secretion of leptin by adipose tissue may lead to an increase in the production of cytokines and MMPs, thereby inducing chondrocyte injury and apoptosis [8]. Currently, although metabolic OA has received widespread attention as a new subtype of OA, evidence on its pathogenesis is still limited.

DDIT4, also known as regulated in development and DNA damage response 1 (REDD1), is an inhibitor of rapamycin target protein 1 (mTOR1) and plays a critical role in hypoxia, cell damage, and various stress processes [9]. Currently, many studies have demonstrated the regulatory role of DDIT4 in inflammatory diseases. For example, the autophagy pathway regulated by DDIT4 promoted thrombotic inflammation and fibrosis in human systemic lupus erythematosus through extracellular trapping neutrophils modified with tissue factor and interleukin-17A [10]. Importantly, studies found that DDIT4 regulated mitochondrial function in articular cartilage and was associated with the severity of OA [11]. In addition, DDIT4 is involved in the insulin sensitivity of hyperlipidemia patients and the pathological process of diabetes induced nephritis [12,13]. However, there is no evidence about whether DDIT4 can be used as a diagnostic indicator of DOA and its regulatory role in the pathological progression of DOA at present.

The purpose of this study was to explore the abnormal expression of DDIT4 in the pathological process of DOA and analyzed the correlation between the abnormal expression of DDIT4 and diabetes OA, further explored whether DDIT4 could be used as a key regulatory factor in the pathological development of diabetes-induced OA, and explored its potential regulatory mechanism, so as to provide theoretical basis and biological target for the diagnosis and treatment of DOA.

2. Materials and methods

2.1. Samples

In this study, we recruited OA patients and non-OA subjects who undergo total knee replacement surgery in clinical practice and collected clinical and pathological information of all OA patients, as well as blood samples, synovial fluid samples, and synovial tissue samples from both OA patients and non-OA subjects. According to the collected clinical information of OA patients and the blood glucose indicators detected with their serum samples, OA patients were divided into two groups: diabetic OA (fasting blood glucose $\geq$ 7.0 mmol/L, 2-h postprandial blood glucose $\geq$ 11.1 mmol/L, excluding diabetes patients with BMI obesity (BMI > 30) and non-diabetic OA. The study was reviewed and approved by the Institutional Medical Ethics Committee of Henan Provincial People's Hospital, Zhengzhou University People's Hospital. According to the Declaration of Helsinki, the patients whose samples were used for this research provided informed consent.

2.2. Cell culture

We isolated human FLSs from synovial tissue using collagenase treatment. Separated primary FLSs were incubated in with DMEM and 10% fetal bovine serum at 37°C and 5% CO_2. Primary FLSs were treated with LPS (100 ng/mL, 24 h) or high glucose (HG, 25 mM).

2.3. Transfection

The pcDNA3.1-DDIT4 (pc-DDIT4) were designed and synthesized by GenePharma (Shanghai, China). The pcDNA3.1-DDIT4 or pcDNA3.1-scramble together with Lipofectamine 2000 (Beyotime, Shanghai, China) were used for the cell transfection according to the manufacturer's protocol. The FLSs were harvested following a 48 h-transfection for subsequent studies.

2.4. CCK-8

Cells were suspended at a concentration of 2×10^4 cells/well and evenly distribute them in a 96-well plate. After a 72 h-incubation, add 20 μL of CCK-8 (Beyotime, Beijing, China). Following 4 h cell culture, add 150 μL of DMSO (Dojindo, Kumamoto, Japan) to each well and shake for 10 min. Finally, measure the absorbance at 450 nm using an enzyme meter.

2.5. Cell apoptosis

Apoptosis assays were performed using Annexin V-FITC Apoptosis Assay Kit (Takara, Shiga, Japan) according to the manufacturer's protocol. The treated cells were digested with trypsin, washed 3 times with PBS and then resuspended in 100 μL binding buffer. Subsequently, Annexin-V and PI was added to the cell suspension and incubate for 15 min. And the apoptosis rate was detected using an FCM flow cytometer (BD Bioscience, CA, USA) according to the instructions.

2.6. Wound healing assay

Cells were cultivated to 70% ~ 80% confluence and form a “wound” using sterile lance tip (Axygen Biosciences, CA, USA). Then, the culture medium was washed with PBS (Beyotime, Shanghai, China) to remove the cell debris produced during the wound-making process. Subsequently, migration observation and recording were conducted using a microscope.

2.7. Transwell assay

Cells were cultured in a serum-free DEME medium for 48 h. Trypsin (Thermo Fisher Scientific, MA, USA) was utilized to digest cells and prepare cell suspensions. The cells were evenly distributed in the upper chamber of the Transwell and the medium containing serum was supplemented into the lower chamber. Twenty-four hour later, cells migrated in the lower chamber were fixed with formaldehyde (Aladdin, Shanghai, China) and stained with crystal violet solution for 30 min. The migrated cells were photographed and analyzed by using a light microscopy (Bio-Rad, CA, USA).

2.8. RT-qPCR

The total RNA was extracted by using TRIzol kits (Beyotime, Shanghai, China). cDNA was synthesized using a reverse transcription kit (Takara, Shiga, Japan) according to the manufacturer's protocol. Relative changes in gene expression were calculated by the help of the $2^{-\Delta\Delta Ct}$ method.

2.9. Western blot

Total cellular proteins were extracted and protein concentrations were determined using BCA Protein Assay Kit (Beyotime,

Shanghai, China). Equal amounts of proteins were isolated by 10% SDS-PAGE and transferred onto PVDF membranes (Millipore, MA, USA). Following being blocked for 2 h, the membranes were cultured with the indicated antibodies overnight at 4°C. The primary antibodies as follows: anti-DDIT4 (1:1000, Abcam), anti-MMP3 (1:800, Abcam), anti-MMP13 (1:800, Abcam), anti-Aggrecan (1:800, Abcam), anti-COL3A1 (1:800, Abcam), anti-HK2 (1:1000, Abcam), anti-PKM2 (1:1000, Proteintech), anti-PFKP (1:1000, Abcam), anti-GAPDH (1:2000, Abcam). After washing with TBST solution (Takara, Shiga, Japan), the membranes were incubated with secondary antibody (1:5000) for 1 h.

2.10. Assessment of extracellular flux

An XF24 Extracellular Flux Analyzer (Seahorse Bioscience Inc., Billerica, MA) was used to determine the extracellular acidification rate (ECAR) and oxygen consumption rate (OCR). Wave 2.6.0 software was used for the analysis of Seahorse data.

2.11. Statistical analysis

All experiments were replicated across a minimum of three distinct groups, and the findings were expressed as "mean ± SEM". When comparing just two groups, we relied on the *t*-test. However, for assessing disparities among multiple groups, we adopted one-way ANOVA followed by Tukey's post hoc test. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. The expression level of DDIT4 was significantly reduced in OA patients and FLSs

Firstly, we collected blood, synovial fluid, and synovial tissue samples from OA patients and non-OA subjects. The results showed that the mRNA expression level of DDIT4 in serum, synovial fluid and synovial tissue of the OA group was significantly decreased than that of the control group (Fig. 1A–C). Subsequently, we classified OA patients and further measured the expression level of DDIT4. The results showed that as the patient's condition worsened, the mRNA expression levels of DDIT4 in serum, synovial fluid, and synovial tissue gradually decreased (Fig. 1D–F). In addition, we isolated human FLSs from synovial tissues. RT-qPCR and Western blotting analysis showed that the mRNA and protein expression levels of DDIT4 in primary FLSs of the OA group were significantly higher than those in the control group (Fig. 1G and H). In addition, we treated FLSs with LPS and detected the expression of DDIT4. The results showed that LPS significantly increased the expression level of DDIT4 in primary FLSs (Fig. 1I and J).

3.2. Overexpression of DDIT4 inhibited overactivation, migration, and invasion of FLSs as well as alleviated chondrocyte injury

Firstly, an overexpression vector of DDIT4 (pcDNA3.1-DDIT4) was constructedand and transfected into FLSs. The results showed that the 0.5 μg pc-DDIT4 overexpression vector significantly increased the expression level of DDIT4 in LPS-induced FLSs. The transfection efficiency of the 0.2 μg pc-DDIT4 overexpression vector was lower compared to the 0.5 μg pc-DDIT4 overexpression vector (Fig. 2A and B). Therefore, the 0.5 μg pc-DDIT4 overexpression vector was used in subsequent experiments. Further research results indicated that LPS significantly enhanced the cell viability, migration and invasion ability of FLSs, while overexpression of DDIT4 significantly reduced the cell viability, migration and invasion of FLSs (Fig. 2C–E). As expected, the mRNA and contents of inflammatory factors (IL-1β, IL-6 and TNF-α), ROS production, and iNOS content were significantly increased in the LPS group compared to the untreated group, while the levels of these indicators in the LPS + pc-DDIT4 group were significantly lower than those in the LPS + pcDNA-3.1 group (Fig. 2F–I). Subsequently, we co-cultured primary FLSs with chondrocytes (ATDC5) *in vitro* to analyze the regulatory effect of DDIT4 on chondrocyte abnormalities during OA progression. The results showed that LPS significantly reduced the activity of ATDC5 cells and increased the apoptosis, while overexpression of DDIT4 increased the activity of ATDC5 cells and decreased the apoptosis (Fig. 2J and K). In addition, LPS induced the expression of Aggrecan, COL3A1, MMP3 and MMP13, as well as overexpression of DDIT4 in FLSs inhibited the blocking effect of LPS on Aggrecan, COL3A1, MMP3 and MMP13 protein expression in ATDC5 cells (Fig. 2L).

3.3. The DDIT4 expression was downregulated in DOA patients and was associated with DOA

Next, we divided the collected OA patients into non-diabetes type OA (ND-OA) and diabetic OA (DOA) according to the blood glucose levels. Firstly, we analyzed the fasting blood glucose, total cholesterol, triglycerides, and insulin levels of patients in the non-OA (control subjects), ND-OA, and D-OA group. The results showed that the fasting blood glucose and triglyceride content in the ND-OA group were slightly higher than those in the control group, but the difference was not significant. There was no significant difference in total cholesterol content between the ND-OA and D-OA group, but the insulin content in the ND-OA group was significantly lower than that in the control group. In addition, fasting blood glucose levels, total cholesterol, and triglyceride content were significantly increased in the D-OA group compared with the ND-OA group, while insulin content was significantly reduced (Fig. 3A–D). Besides, the results showed that the content of DDIT4 was the lowest in the synovial fluid of the D-OA group, and the highest in the control group (Fig. 3E). The correlation analysis results showed that the expression level of DDIT4 in synovial fluid was significantly negatively correlated with fasting blood glucose, total cholesterol and triglycerides, as well as positively correlated with insulin content (Fig. 3F–I). Further research has found that the mRNA expressions of inflammatory factors (IL-1β, IL-6 and TNF-α) in synovial tissue of patients with osteoarthritis were significantly increased and negatively correlated with the expression of DDIT4 (Fig. S1).

3.4. DDIT4 inhibited HG-induced overactivation, migration and invasion of FLSs, and alleviated HG-induced chondrocyte injury

FLSs were treated with high glucose medium and transfected with DDIT4 overexpression plasmid (pcDNA3.1-DDIT4). RT-qPCR and Western blotting results showed that high glucose reduced DDIT4 expression in FLSs, and transfection of pc-DDIT4 overexpression vector reversed this effect (Fig. 4A and B). In addition, HG significantly promoted the cell viability, migration, and invasion of FLSs, while further overexpression of DDIT4 significantly inhibited the cell viability, migration, and invasion of FLSs (Fig. 4C–E). Similarly, HG promoted the mRNA and contents of IL-1β, IL-6 and TNF-α, as well as the synthesis of ROS and iNOS in FLSs. Overexpression of DDIT4 significantly inhibited the mRNA and contents of IL-1 β, IL-6 and TNF-α, as well as the content of ROS and iNOS (Fig. 4F–I) in HG-induced FLSs. We co-cultured primary FLSs and chondrocytes (ATDC5). The cell viability and the expression of Aggrecan, COL3A1, MMP3 and MMP13 were significantly lower in the HG group than those in the untreated group. The cell viability and protein expression of Aggrecan, COL3A1, MMP3 and MMP13 in

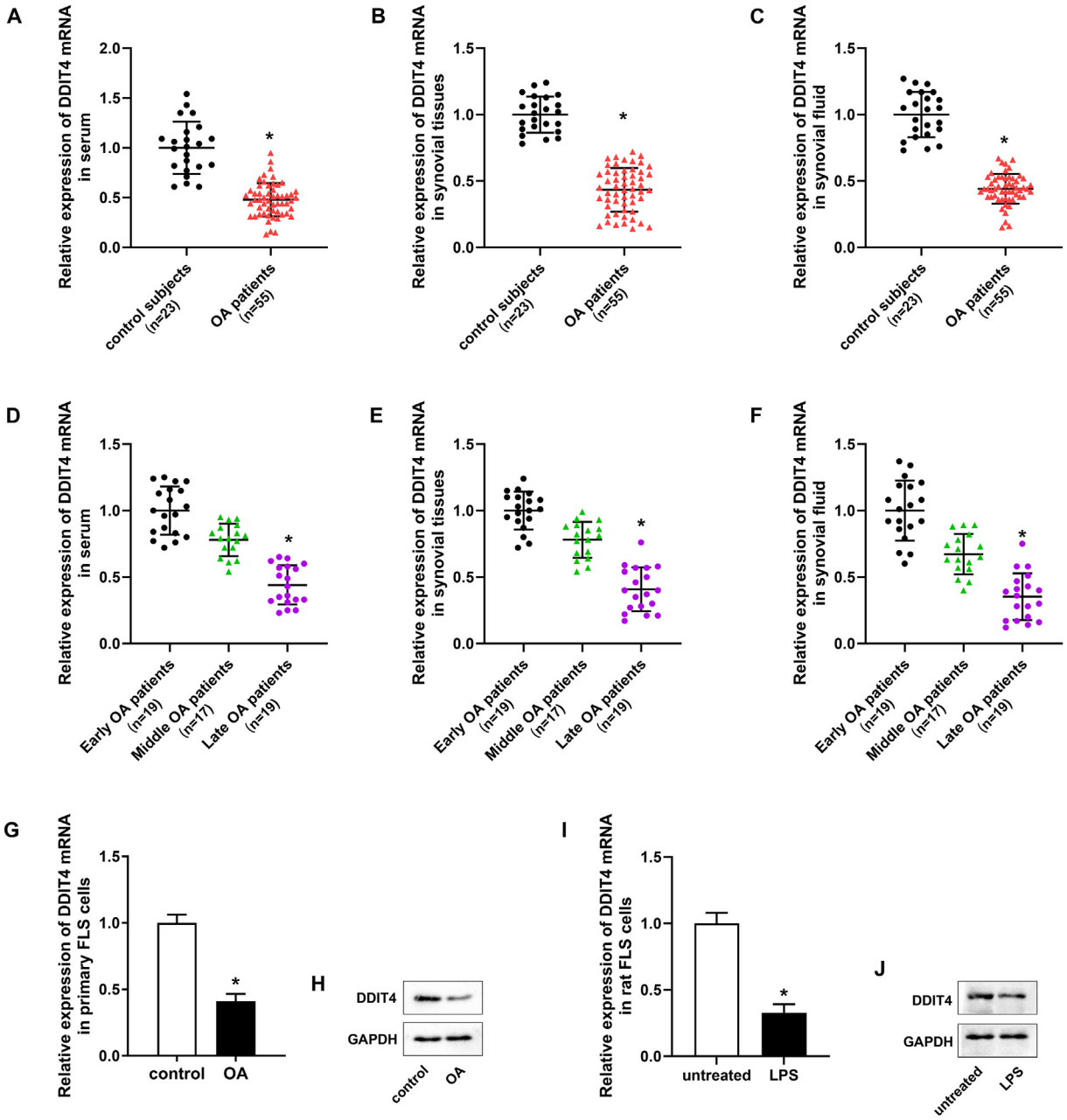

Fig. 1. The expression level of DDIT4 is significantly reduced in OA patients and cells. Blood, synovial fluid and synovial tissue samples from OA patients and non-OA subjects were collected. (A) The mRNA expression of DDIT4 in serum from OA patients and non-OA subjects. (B) The mRNA expression of DDIT4 in synovial tissue from OA patients and non-OA subjects. (C) The mRNA expression of DDIT4 in synovial fluid from OA patients and non-OA subjects. (D) The mRNA expression of DDIT4 in serum from early, middle and late OA patients. (E) The mRNA expression of DDIT4 in synovial tissue from early, middle and late OA patients. (F) The mRNA expression of DDIT4 in synovial fluid from early, middle and late OA patients. (G) The mRNA expression of DDIT4 in FLSs isolated from synovial tissue. (H) The protein expression of DDIT4 in FLSs isolated from synovial tissue. FLSs were treated with LPS (100 ng/mL) for 24 h. (I) The mRNA expression of DDIT4 in FLSs. (J) The protein expression of DDIT4 in FLSs. *$P < 0.05$ vs. control subjects or control or untreated group.

the HG + pc-DDIT4 group were significantly higher compared to the HG group (Fig. 4J, L). Moreover, the apoptosis of ATDC5 chondrocytes was significantly higher in the HG group, while the apoptosis in the HG + pc-DDIT4 group was significantly reduced compared to the HG group (Fig. 4J).

3.5. *DDIT4 alleviated HG-induced FLSs inflammation and functional abnormalities by regulating glycolysis*

Further research has found that high glucose promoted lactate production, glucose uptake, and LDH activity in FLSs, while increasing extracellular acidification and oxygen consumption rates. Additionally, the expression levels of HK2, PFKP and PKM2 were significantly upregulated by high glucose (Fig. 5A–F). The overexpression of DDIT4 significantly reduced lactate production, glucose uptake, and LDH activity in FLSs, as well as down-regulated ECAR, OCR, and protein expression levels of HK2, PFKP and PKM2 (Fig. 5A–F). HG significantly promoted the cell survival, migration, invasion, mRNA and content of IL-1β, IL-6, as well as TNF - α in FLS, while Cyto-B and 3BrPA significantly inhibiting HG's promotion of the above indicators (Figs. S2A–S2E). Furthermore, HG significantly increased lactate production, glucose uptake, and LDH activity in

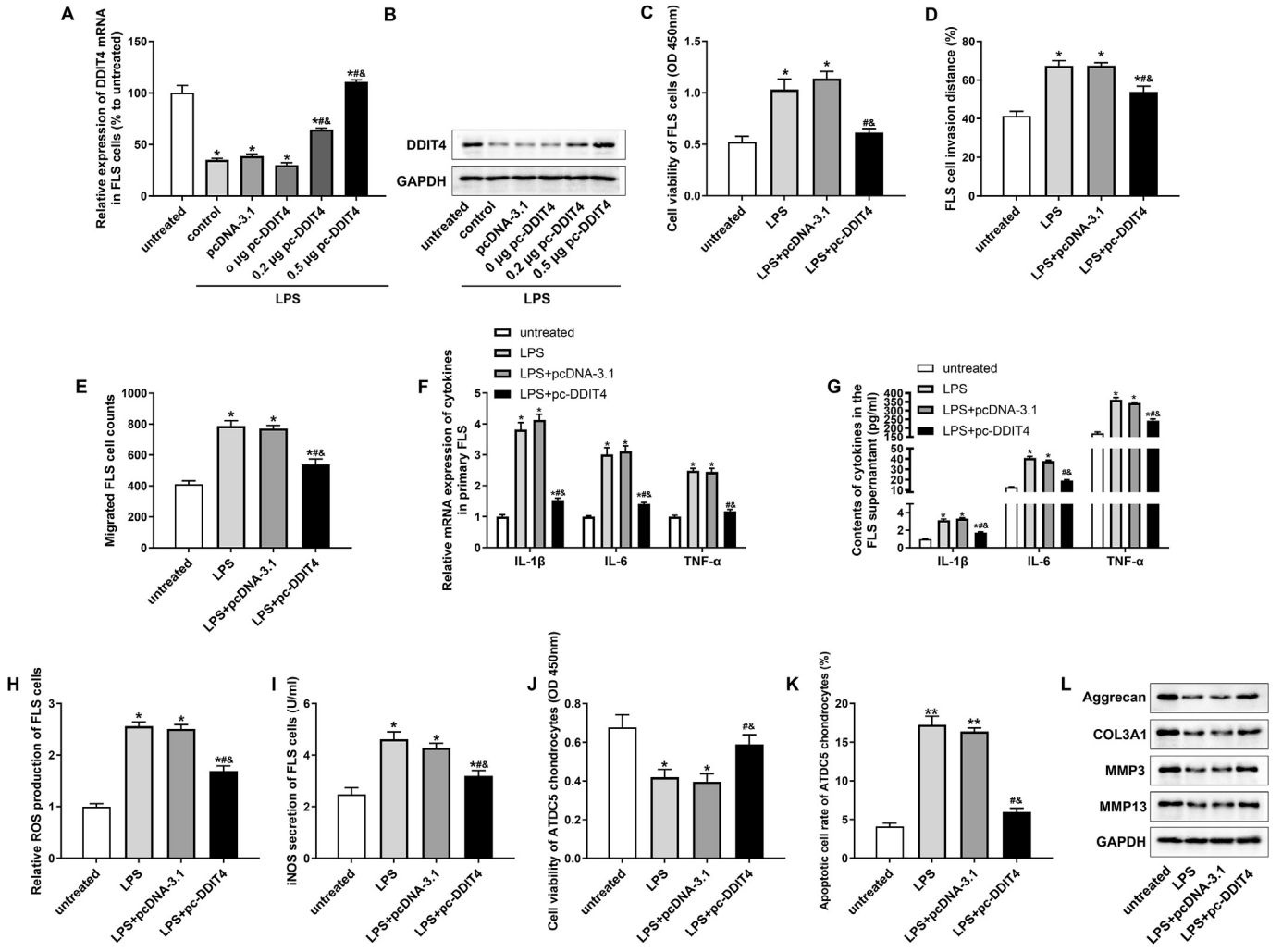

Fig. 2. Overexpression of DDIT4 inhibits overactivation, migration and invasion of FLSs as well as alleviates chondrocyte injury. FLSs transfected with 0.5 μg or 0.2 μg DDIT4 overexpression vector (pc-DDIT4), and treated with LPS (100 ng/mL) for 24 h. (A–B) The mRNA and protein expression of DDIT4 in FLSs after transfection pcDNA3.1-DDIT4. (C) The cell viability of FLSs. (D) The cell invasion of FLSs. (E) The cell migration of FLSs. (F) The mRNA expression of IL-1β, IL-6 and TNF-α of FLSs. (G) The contents of IL-1β, IL-6 and TNF-α of FLSs. (H) The ROS production of FLSs. (I) The contents of iNOS of FLSs. FLSs and ATDC5 cells were co-cultured. (J) The cell viability of ATDC5 cells. (K) The cell apoptosis of ATDC5 cells. (L) The protein expression of Aggrecan, COL3A1, MMP3 and MMP13 in ATDC5 cells. $^{*}P < 0.05$ vs. untreated group; $^{**}P < 0.01$ vs. untreated group; $^{\#}P < 0.05$ vs. LPS group; $^{\&}P < 0.05$ vs. LPS + pcDNA3.1 group.

FLSs, and promoted protein expression levels of HK2, PFKP, and PKM2, as well as ECAR and OCR. Cyto-B and 3BrPA (the glycolytic inhibitors) have opposite effects on the above indicators (Figs. S2F–S2K). Cyto-B and 3BrPA had no significant effect on HG-induced downregulation of DDIT4 (Fig. 5G and H). Importantly, the apoptosis of ATDC5 cells in the HG + Cyto-B and HG+3BrPA groups were significantly lower than that in the HG group (Fig. 5I). Interestingly, high glucose promoted the protein expression of aggrecan, COL3A1, MMP3 and MMP13 in ATDC5 cells, while Cyto-B or 3BrPA reversed the promoting effect of high glucose (Fig. 5I and J).

4. Discussion

DDIT4 is upregulated under stress conditions such as hypoxia, oxidative stress, and DNA damage, affecting processes such as cellular autophagy, apoptosis, and energy stress [14]. Research has shown that the expression of DDIT4 induced cell cycle arrest, inhibited cell growth, and participated in important physiological processes such as regulating cell death pathways [15]. Currently, studies have demonstrated the regulatory role of DDIT4 in inflammatory diseases. Research reports that DDIT4 is involved in the regulation of lumbar disc herniation injury and neuroinflammation [15,16]. Importantly, Chen et al. found that DDIT4 can be used as a candidate gene to predict the probability of OA by analyzing the glycolysis pattern of synovial samples from OA patients [17]. Therefore, we speculated that DDIT4 may be involved in the progression of OA. Our study found that the expression level of DDIT4 was downregulated in OA patient's synovial samples and LPS-induced FLSs, and DDIT4 overexpression inhibited the secretion of inflammatory factors and alleviated the pathological process of OA. However, previous research reported that DDIT4 promoted macrophage inflammation by regulating glucose uptake, glycolysis, and oxidative phosphorylation [18], which is contrary to our findings. We speculate that DDIT4 may play different roles in different diseases or cells. In addition, the regulatory mechanisms and pathways involved in diseases are complex and may also have different regulatory effects.

OA is the most common degenerative joint disease, and FLSs play an important role in the pathogenesis of OA. FLSs is a special type of cell found within the synovial membrane of joints (a soft

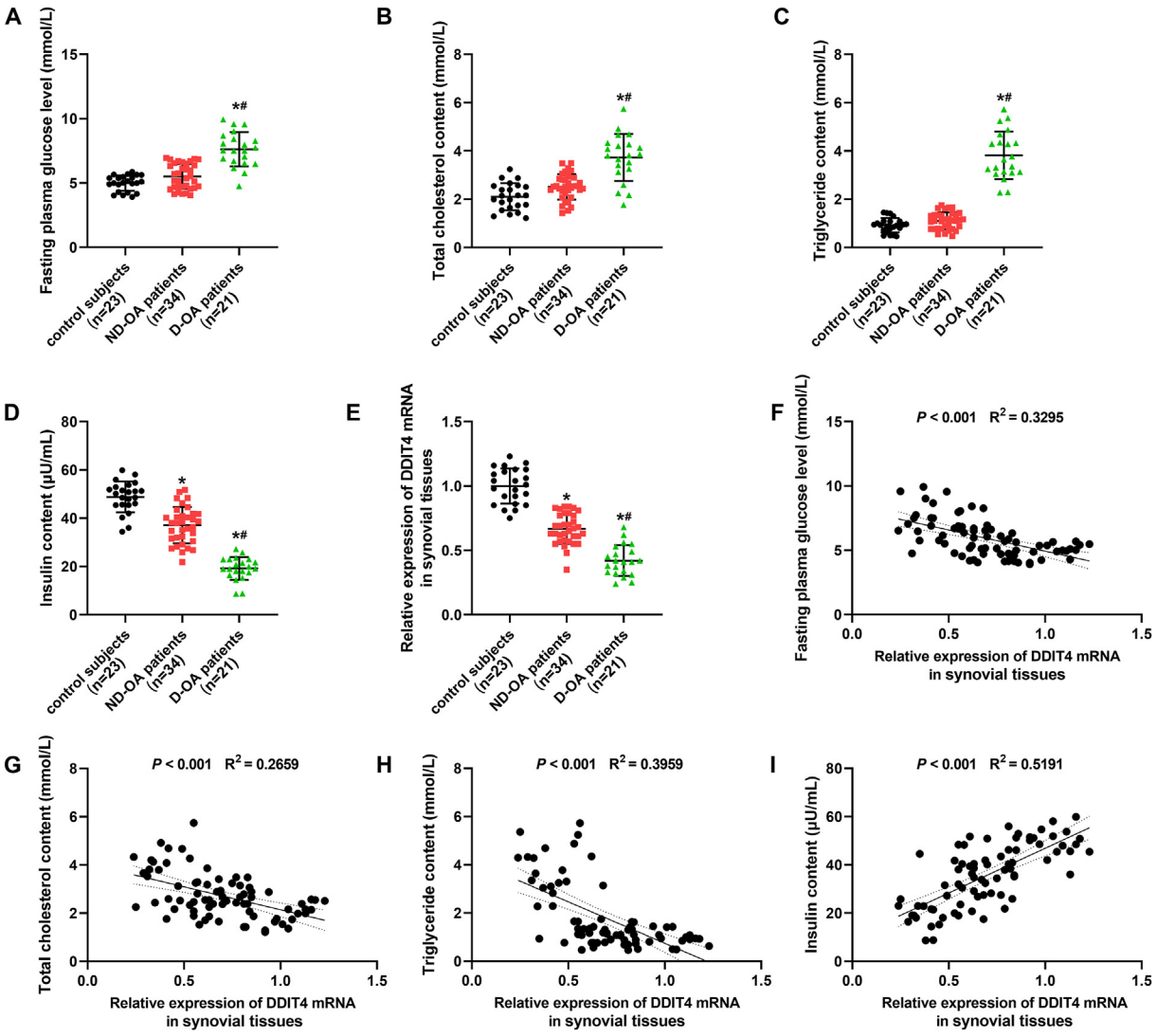

Fig. 3. The DDIT4 expression was downregulated in DOA **patients and was associated with DOA.** OA patients were divided into two groups: non-diabetes type OA (ND-OA) and diabetic OA (D-OA). Fasting blood glucose (A), total cholesterol content (B), triglycerides content (C), and insulin content (D) in the control subjects, ND-OA and D-OA groups. (E) The DDIT4 content in synovial fluid from the control subjects, ND-OA and D-OA patients. The correlation between the mRNA expression of DDIT4 in synovial fluid and fasting blood glucose (F), total cholesterol content (G), triglycerides content (H), and insulin content (I). *$P < 0.05$ vs. control subject group.

connective tissue that lubricates the joints and minimizes wear) [19]. Activated FLSs exhibit abnormal migration, invasion, and secretion of pro-inflammatory cytokines and chemokines [20]. Research has found that the secretion of inflammatory FLSs derived exosomes was increased in OA and exacerbated the inflammatory response by enhancing macrophage glycolysis and M1 polarization, further promoting the progression of OA [21]. In addition, high mobility group box 1 (HMGB1) promotes the production of pro-inflammatory cytokines in FLSs. Meanwhile, researchers found that HMGB1 also promoted the generation of VEGF in FLS, indicating that promoting angiogenesis in FLSs further exacerbates joint inflammation [22]. The above evidence suggested that targeting FLSs may be a new treatment for OA.

Interestingly, recent reports suggested that targeted glucose metabolism reprogramming is a promising strategy for treating OA [23]. A recent study suggests that increased glycolytic metabolism in FLSs contributes to synovitis and joint injury in rheumatoid arthritis [24]. Furthermore, Damerau et al. reported metabolic changes induced by synovitis in FLS of OA patients. Inhibiting metabolic reprogramming of FLS reduces FLS proliferation and secretion of inflammatory cytokines. The study provides new mechanistic insights into the importance of FLS in the pathogenesis of OA [23]. Garcia et al. found that the balance of glycolysis and oxidative phosphorylation in FLS of RA patients shifted towards glycolysis, and in a mouse inflammatory arthritis model, there was an increase in glucose uptake and expression levels of glycolysis related proteins. Inhibition of glycolysis *in vivo* can significantly reduce the severity of arthritis in mouse models, indicating that the glycolysis of FLSs accelerates the progression of osteoarthritis [25].In this study, we found that glycolytic inhibitors alleviate FLSs inflammation and abnormal chondrocyte function. Therefore, our study further supports the viewpoint that increased glycolytic metabolism of FLSs leads to OA joint inflammation.

Recent studies have reported that DDIT4 plays a crucial role in the pathological progression of related diseases by regulating cellular glycolysis processes [26]. There are reports that DDIT4 promotes macrophage inflammation by regulating glucose uptake, glycolysis, and oxidative phosphorylation [18]. Moore et al. reported that DDIT4 can be a pathogenic factor of oxidative stress in diabetes retinopathy [27]. Moreover, DDIT4 is involved in the

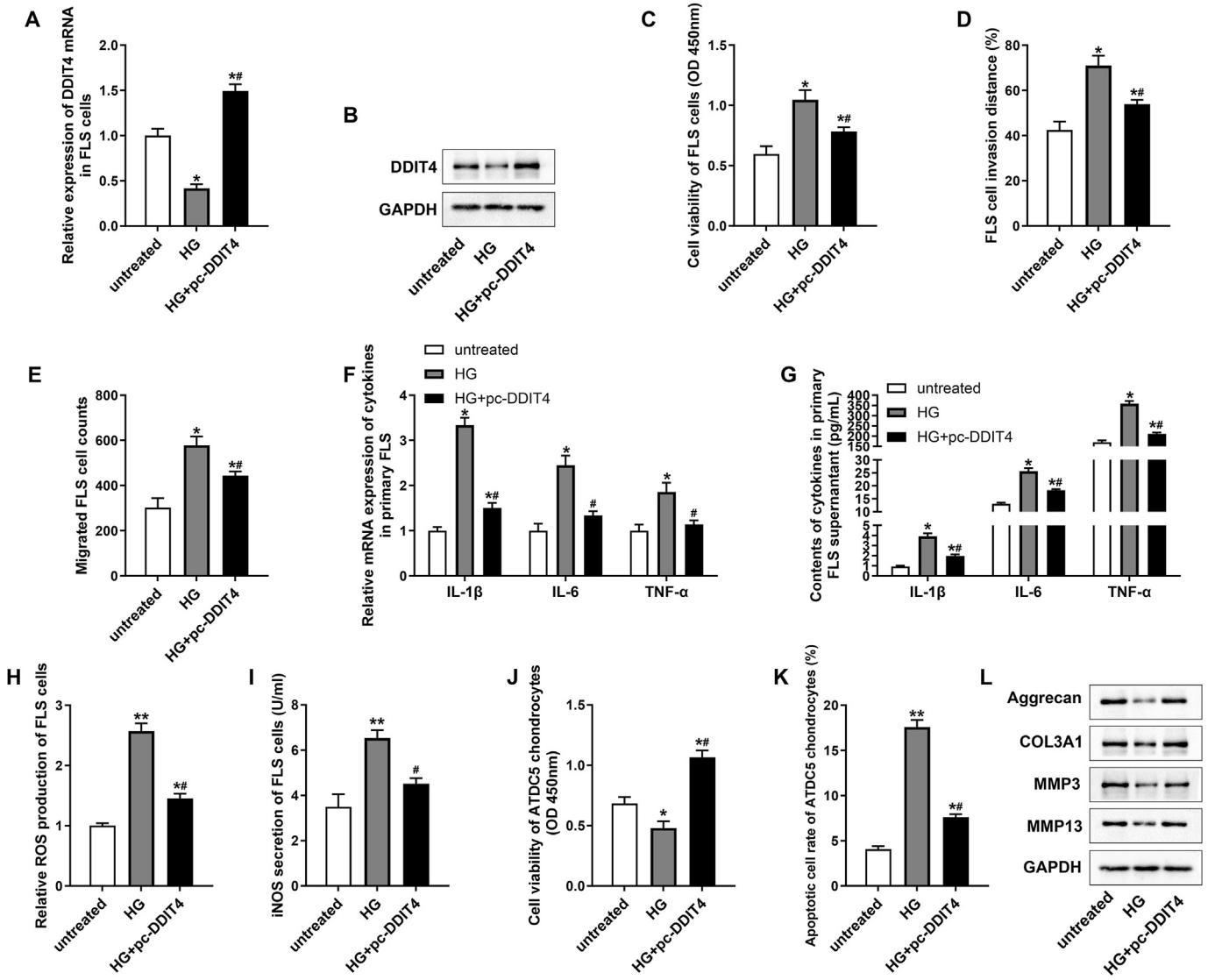

Fig. 4. Overexpression of DDIT4 inhibits HG-induced overactivation, migration, and invasion of FLSs, and alleviates HG-induced chondrocyte injury. FLSs were treated with high glucose medium and transfected with DDIT4 overexpression plasmid (pcDNA3.1-DDIT4). (A) The mRNA expression of DDIT4 a in HG-induced FLSs. (B) The protein expression of expression of DDIT4 a in HG-induced FLSs. (C) The cell viability of FLSs. (D) The cell invasion of FLSs. (E) The cell migration of FLSs. (F) The mRNA expression of IL-1β, IL-6 and TNF-α of FLSs. (G) The contents of IL-1β, IL-6 and TNF-α of FLSs. (H) The ROS production of FLSs. (I) The contents of iNOS of FLSs. FLSs and ATDC5 cells were co-cultured. (J) The cell viability of ATDC5 cells. (K) The cell apoptosis of ATDC5 cells. (L) The protein expression of Aggrecan, COL3A1, MMP3 and MMP13 in ATDC5 cells. $^{*}P < 0.05$ vs. untreated group; $^{**}P < 0.01$ vs. untreated group; $^{\#}P < 0.05$ vs. HG group.

insulin sensitivity of hyperlipidemia patients and the pathological process of diabetes induced nephritis [13]. At present, there is no report on whether DDIT4 can be used as a diagnostic indicator of DOA and its regulatory role in the pathological progress of DOA. Our study found that DDIT4 participates in HG-induced overactivation, inflammation of FLSs and chondrocyte injury by regulating glycolysis processes. The above research shows that DDIT4 plays an important role in the development of diabetes and its complications, including OA.

Diabetes is characterized by insulin mechanism disorder, which leads to hyperglycemia, chronic inflammation, and other complications, including OA [28]. According to previous studies, patients with diabetes and OA have common characteristics, such as obesity, hypertension and dyslipidemia [29]. Although the causal relationship between T2DM and OA is still unclear, the correlation between the two diseases has been confirmed. Louati et al. conducted a meta-analysis of 49 studies involving over 1 million participants and found a significant correlation between OA and T2DM [30]. Currently, metabolic OA as a new subtype of OA has received widespread attention [31]. It is reported that joint injury caused by DOA by increasing oxidative stress and proinflammatory factors [32]. A study reported that insulin dependent phosphorylation of insulin receptors and serine/threonine kinase Akt were reduced in f FLSs of DOA patients, confirming the existence of insulin resistance in the synovium of DOA patients [33]. In addition, diabetes induce inflammation by increasing blood glucose concentration through insulin resistance, which is a common pathological mechanism of many metabolic related diseases, including T2DM [34]. In this study, the results showed that compared with non-diabetes OA patients, fasting blood glucose levels, total cholesterol, and triglyceride content were significantly increased in DOA patients, while insulin content was significantly reduced. Therefore, prevention of diabetes may be a feasible measure to alleviate OA.

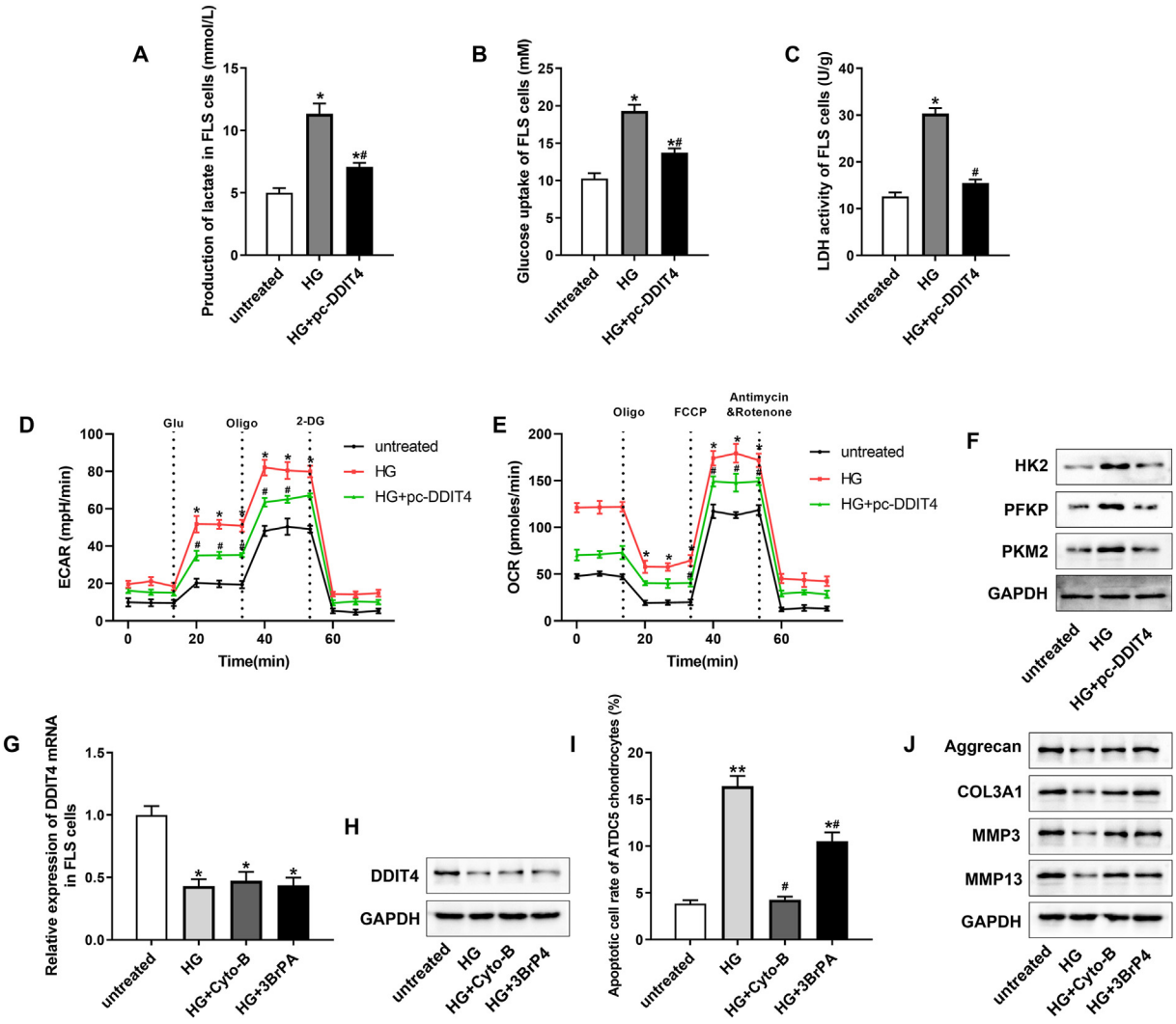

Fig. 5. DDIT4 alleviates HG-induced FLSs inflammation and functional abnormalities by regulating glycolysis. FLSs were treated with high glucose medium and transfected with DDIT4 overexpression plasmid (pcDNA3.1-DDIT4). (A) The lactate production in FLSs. (B) The glucose uptake in FLSs. (C) The LDH activity of FLSs. (D) The extracellular acidification rate (ECAR) of FLSs. (E) The oxygen consumption rate (OCR) of FLSs. (F) The protein expression of HK2, PFKP and PKM2 in FLSs. FLSs were treated with glycolysis inhibitors Cyto-B and 3BrPA. (G) The protein expression of DDIT4 in FLSs. FLSs were treated with HG or glycolysis inhibitors, and the supernatant was taken to continue culturing ATDC5 cells. (H) The protein expression of DDIT4 in FLSs. (I) The cell apoptosis of ATDC5 cells. (J) The protein expression of Aaggrecan, COL3A1, MMP3 and MMP13 in ATDC5 cells. $^{*}P < 0.05$ vs. untreated group; $^{**}P < 0.01$ vs. untreated group; $^{\#}P < 0.05$ vs. HG group.

In conclusion, DDIT4 was downregulated in OA patient's synovial samples and FLS-induced cells, and participated in the progression of OA by regulating glycolytic processes, inducing HG-induced FLSs overactivation and inflammation, as well as FLSs-exerted chondrocyte injury. In addition, abnormal expression of DDIT4 is associated with DOA. It can be concluded that DDIT4 can be a key regulatory factor for the pathological development of DOA.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Henan Provincial People's Hospital, Zhengzhou University People's Hospital. Informed consents were provided by every patient in accordance with the Declaration of Helsinki.

Consent for publication

The authors participated in this study were all consented for the publication.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author contributions

Yunke Liu contributed to the study conception and design. Cheng Cheng and Yonghui Dong contributed to Data collection and Data analysis. Chao Tang and Jia Zheng contributed to Formal analysis and Software. Shuo Qiang contributed to the writing of first draft of the manuscript. All authors commented on previous versions of the manuscript, read and approved the final manuscript.

Funding

This work was supported by the Henan Province Science and Technology Research Project [grant numbers: 232102310076],

Henan Province Medical Science and Technology Research Project [grant numbers: LHGJ20210004].

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Abbreviations

OA	osteoarthritis
DDIT4	DNA damage-inducible transcript 4 protein
DOA	diabetic osteoarthritis
FLSs	fibroblast-like synoviocytes
HG	high glucose
TNFs	tumor necrosis factors
IL	interleukins
MMPs	matrix metalloproteinases
REDD1	DNA damage response 1
mTOR1	rapamycin target protein 1
HMGB1	high mobility group box 1

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2025.02.017.

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