SCD1 deficiency exacerbates osteoarthritis by inducing ferroptosis in chondrocytes

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Background: Osteoarthritis (OA) is a severe joint disease that causes cartilage destruction and mobility loss. Abnormal fatty acid metabolism of chondrocytes plays a role in OA development. Stearoyl-CoA desaturase (SCD1) is a rate-limiting enzyme in the anabolism of unsaturated fatty acids. This study aimed to investigate the role of the SCD1 protein in the degenerative process of OA.

Methods: The GSE176199 gene expression profile dataset was analyzed by Gene Set Enrichment Analysis (GSEA). An animal model of OA was established using C57BL/6J wild-type (WT) (n=40) and SCD1 knockout (SCD1-KO) (n=20) mice. The histological scoring method of the Osteoarthritis Research Society International (OARSI) was used to quantify the degree of cartilage degeneration. The expression of SCD1 protein and relevant ferroptosis indicators were evaluated.

Results: The GSEA analysis showed that unsaturated fatty acid synthesis was inhibited in human OA chondrocytes. Meanwhile, the expression of SCD1 protein was significantly reduced in human OA articular cartilage. SCD1-KO mice exhibited early OA and accelerated cartilage loss after destabilization of medial meniscus (DMM)-induced OA. Furthermore, we found that the SCD1-PPARG axis regulates articular cartilage homeostasis via a mechanism involving the induction of ferroptosis-related gene expression in ATDC5 chondrocytes.

Conclusions: SCD1 deficiency exacerbates OA by inducing ferroptosis in chondrocytes.

Keywords: Articular cartilage; stearoyl-CoA desaturases; monounsaturated fatty acids; osteoarthritis (OA); ferroptosis

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Introduction

Osteoarthritis (OA) is the most common degenerative joint disease in the world and is also the leading cause of joint disability in the elderly (1). The main pathological feature of OA is articular cartilage degeneration with a variety of pathological changes, including cartilage loss, subchondral bone sclerosis, and synovial inflammation (2). However, the molecular mechanisms underlying the pathogenesis of OA are mostly unknown. Although OA is reported to be associated with unsaturated fatty acid metabolism disorders (3,4), the significance of different Stearoyl-CoA desaturases in the pathogenesis of OA has not been fully illustrated.

Stearoyl-CoA desaturase (SCD1) is a rate-limiting enzyme in the anabolism of unsaturated fatty acids that transforms saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA). Synthetic MUFA and SFA bind to a wide range of lipid classes, including triglycerides (TG) and phospholipids (PL), which are both essential components of cell membranes (5). SCD1 is considered an essential regulator of lipid homeostasis, particularly in the liver and adipose tissue. The regulation of SCD1 activity has been proven to be associated with the development of metabolic syndrome and inflammatory states (6,7). In addition, SCD1 catalytic product MUFA to compete with polyunsaturated fatty acids (PUFA) for incorporation into phospholipids on the cell membrane and exhibit anti-lipid peroxidation effects in modulating the *de novo* lipogenesis network (8). Lastly, SCD1 activation has been demonstrated to enhance the Akt pathway, presumably promoting the transcription of lipogenic enzymes, such as the sterol regulatory element binding protein (SREBP) family members, and thereby stimulating lipid synthesis and membrane formation (9). These findings imply that SCD1 is critical in maintaining cell membrane structural components and stability. So, might SCD1 affect chondrocyte destiny and thus the incidence and progression of OA via modulating lipid balance and cell membrane components?

Ferroptosis is a type of programmed cell death that induces lipid peroxidation of highly abundant PUFA on the cell membrane under the catalysis of ferrous iron or ester oxygenase and cell death (10). It is also associated with neurodegenerative diseases, cardiovascular diseases, and various cancers (11,12). Because chondrocytes are the only cell type in articular cartilage, their survival is critical to maintaining the integrity of the cartilage (13). Ferroptosis in OA has been extensively studied. Increasing data suggests that chondrocyte ferroptosis is linked to the etiology of OA (14). In addition, systemic iron overload and enhanced intracellular iron uptake can promote and aggravate OA in animal models (15). Furthermore, it has also been shown that ferrostatin-1 (Fer-1, a ferroptosis inhibitor) can reduce OA progression (16). Meanwhile, it has been proved that a high level of SCD1 can inhibit the development of ferroptosis in various kinds of cancer cells, including lung, pancreatic, and colorectal cancer (17,18). However, the function of SCD1 in chondrocyte ferroptosis is unknown.

Our previous study revealed that SCD1 protein expression was reduced in human OA cartilage tissue specimens. Also, Gene Set Enrichment Analysis (GSEA) and Gene Ontology (GO) analysis of the GSE176199 dataset showed that unsaturated fatty acid synthesis was inhibited in human OA chondrocytes. While differential genes were enriched on cell membrane components, we hypothesize that deficiency of SCD1 exacerbates OA by inducing ferroptosis in chondrocytes.

Therefore, the present study aimed to investigate the role of the SCD1 protein in the degenerative process of OA. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6630/rc).

Highlight box

Key findings

- Our study demonstrated that SCD1 deficiency exacerbates osteoarthritis by inducing ferroptosis in chondrocytes.

What is known and What is new?

- Abnormal fatty acid metabolism and chondrocyte ferroptosis play a role in osteoarthritis (OA) development. SCD1 inhibits the development of ferroptosis in various kinds of cancer cells.
- Unsaturated fatty acid metabolism is reduced in osteoarthritic chondrocytes. SCD1 deficiency in mouse chondrocytes accelerates cartilage degeneration after injury. The SCD1-PPARG axis regulates ferroptosis in chondrocytes *in vivo*.

What is the implication, and what should change now?

- The SCD1-PPARG axis is a potential target for the treatment of OA.

Methods

Data collection and preparation

The GSE176199 gene expression profile dataset was acquired from the Gene Expression Omnibus (GEO) website; the probes were transformed to the matching

gene names based on the annotation information of the GPL16791 high-throughput sequencer platform (Homo Sapiens) (Illumina HiSeq 2500).

Participant consent and ethical approval

Human cartilage samples were collected from 10 patients who had total knee arthroplasty and 5 patients who had traumatic amputation. Inclusive criteria: (I) age 50–70; (II) patients with knee osteoarthritis, K-L grade 3 or above, who need total knee replacement; (III) or healthy patients who need amputation due to trauma. Exclusion criteria: (I) age over 70 years old or less than 50 years old; (II) arthritis patients with rheumatic, rheumatoid, gout, traumatic arthritis or other autoimmune diseases; (III) have received arthroscopic surgery during the period; (IV) patients with serious medical diseases or chronic immune system diseases. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of Chongqing Medical University's University-Town Hospital (No. 2021.5.28/LL-202133) and informed consent was taken from all the patients.

Animals and animal experiments

8-week-old male WT (C57BL/6J, weight 18–22 g) mice were provided by the Experimental Animal Center of Chongqing Medical University (Chongqing China) and 8-week-old male SCD1-KO (C57BL/6J, weight 18–22 g) mice were acquired from CYAGEN Biotechnology Company (Serial Number: KOCMP-21645-Scd1). The above mice had been randomly assigned and raised at Chongqing Medical University of Sciences Experimental Animal Center. The mice were housed for 12 hours at a constant temperature of 22.2 ℃, relative humidity of 55%±5%, and in a 12h-12h light-dark cycle condition. Animal experiments were performed under a project license (No. 2021.5.28/LL-202133) granted by the ethics board of the University-Town Hospital of Chongqing Medical University, in compliance with national guidelines for the care and use of animals. The WT mice (C57BL/6J, n=20) were randomly grouped and then subjected to sham or right knee medial meniscus surgery-induced OA (DMM model) (19). The WT (C57BL/6J, n=10) and SCD1-KO (C57BL/6J, n=10) mice were randomly grouped and then subjected to right knee DMM surgery. The mice were sacrificed by cervical dislocation 4 and 8 weeks after surgery. The WT (C57BL/6J, n=10) and SCD1-KO (C57BL/6J, n=10) mice were randomly grouped and not specially treated. The mice were sacrificed by cervical dislocation at 6 months of age. The knees were dissected and immediately immersed in paraformaldehyde or temporarily frozen at −80 ℃ before further processing. A protocol was prepared before the study without registration.

Histology and immunohistochemistry

The human and mouse knee joints were fixed for 48 hours in 4% paraformaldehyde, decalcified for 21 days in 20% ethylenediaminetetraacetic acid (EDTA), embedded in paraffin, and sectioned at 5 μm thickness. The sections were stained using Safranin O/Fast Green and hematoxylin and eosin (HE) staining procedures. The histological scoring method of the Osteoarthritis Research Society International (OARSI) was used to quantify the degree of cartilage degeneration (20). Immunohistochemistry was carried out in accordance with the manufacturer's instructions (SP-9000 ZSGB-BIO). The sections were treated for 10 minutes with 3% H_2O_2 to prevent endogenous peroxides. The goat serum was blocked for 1 hour to avoid nonspecific IgG binding. The sections were then incubated with the primary antibody overnight in a humidified atmosphere at 4 ℃. On the next day, the sections were treated for 30 minutes with biotin-associated linkers and then for 1 hour with streptavidin. We used 3,3'-Diaminobenzidine (DAB) as the substrate for the colorimetric detections.

Cell culture and transfection

Lipo8000^{TM} Transfection Reagent was used for the transient transfection experiments, following the manufacturer's recommended protocol (C0533; Beyotime, China). The ATDC5 chondrocytes were seeded in a six-well plate at a density of 2,000 cells/well for 24 hours before transfection. The transfection solution was prepared at a 25:1 ratio of small interfering ribonucleic acid (siRNA) (100 pmol) to Lipo8000^{TM} (4 μL). The nucleic acid sequences of siRNA and negative control (NC) are shown in *Table 1*. The samples were incubated in 5% CO_2 at 37 ℃ for 48 hours. The cultured cells were isolated and used to extract RNA and proteins.

Chondrocyte oxygen stress and apoptosis assays

The ATDC5 chondrocytes were pretreated for

Table 1 The target sequence of siRNA

	Sequence (5' to 3')
siRNA-sense	CCGCGCAUCUCUAUGGAUAUCTT
siRNA-antisense	GAUAUCCAUAGAGAUGCGCGGTT
NC-sense	UUCUCCGAACGUGUCACGUTT
NC-antisense	ACGUGACACGUUCGGAGAATT

NC, negative control.

Table 2 Nucleotide sequences of qPCR primers

Primer name	Sequence (5' to 3')
MMP13-F	TGTTTGCAGAGCACTACTTGAA
MMP13-R	CAGTCACCTCTAAGCCAAAGAAA
GPX4-F	TGTGCATCCCGCGATGATT
GPX4-R	CCCTGTACTTATCCAGGCAGA
SCD1-F	TTCTTGCGATACACTCTGGTGC
SCD1-R	CGGGATTGAATGTTCTTGTCGT
P53-F	CCCCTGTCATCTTTTGTCCCT
P53-R	AGCTGGCAGAATAGCTTATTGAG
GAPDH-F	TGTGTCCGTCGTGGATCTGA
GAPDH-R	TTGCTGTTGAAGTCGCAGGAG

qPCR, quantitative polymerase chain reaction.

24 hours with tumor necrosis factor α (TNF-α) (50 ng/mL) or phosphate buffer saline (PBS), depending on the experimental design. Intracellular reactive oxygen species (ROS) levels were determined using a dihydroethidium (DHE) detection kit as specified by the manufacturer's guidelines (50102ES02; Yeasen, China). To assess cell viability, cell death was determined by an Apoptosis-Hoechst staining kit (C0003; Beyotime, China) after incubation with TNF-α (50 ng/mL) or PBS for 24 hours.

Quantitative reverse transcription PCR (RT-qPCR)

Total RNA was isolated from the ATDC5 chondrocytes using Trizol Reagent (R0016; Beyotime), as directed by the manufacturer. A Prime Script RT Kit was used to reverse transcribe the whole RNA (D7178M; Beyotime). BeyoFast™ SYBR Green qPCR Mix (2X) (D7260; Beyotime) was used for the qPCR in a Light Cycle 480II Real-Time PCR System (Roche). Glyceraldehyde-phosphate dehydrogenase (GAPDH) served as the internal reference for normalization, and the relative expression was calculated using the $2^{-(\Delta\Delta CT)}$ technique. The primer sequences for the qRT-PCR are shown in *Table 2*.

Western Blot

A Protein Extraction Kit (P0013; Beyotime) was used to extract total protein from the cartilage and chondrocytes, which was then measured using the BCA Protein Assay Kit (P0010S; Beyotime). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-Page) was used to separate the protein samples on a polyvinylidene fluoride (PVDF) membrane. The blot was blocked in a 5% bovine serum albumin (BSA) solution for 1 hour at room temperature before being incubated overnight at 4 ℃ with an appropriate dilution of primary antibody. The membrane was then treated for 60 minutes at 37 ℃ with the appropriate secondary antibody. Finally, the enhanced chemiluminescence (ECL) method was applied to detect the protein of interest, and the signal intensity was detected under ECL chemiluminescence equipment.

Statistical analysis

All the data collected in this study were analyzed using GraphPad Prism 9 software (Version 9.3.1). Normally distributed measurement data are expressed as the mean ± standard deviation (SD), while non-normally distributed measurement data are expressed as the median (interquartile range), and the comparisons were examined by Student's *t*-test or Mann-Whitney test (non-parametric distribution). The pretreatment core packages in R (Version 4.1.3, www.r-project.org) were used to normalize the data set and perform the GO analysis, while the GSEA analysis was undertaken with the GSEA app program (Version 4.2.3). A P value <0.05 was considered statistically significant (*, P<0.05, **, P<0.01).

Results

Unsaturated fatty acid metabolism is reduced in osteoarthritic chondrocytes

Abnormal energy metabolism was recently proposed to be correlated with the development of OA (21). To investigate whether metabolic genes were differentially expressed in OA, we downloaded the GSE176199 dataset, which included three normal cartilage stromal cells, three

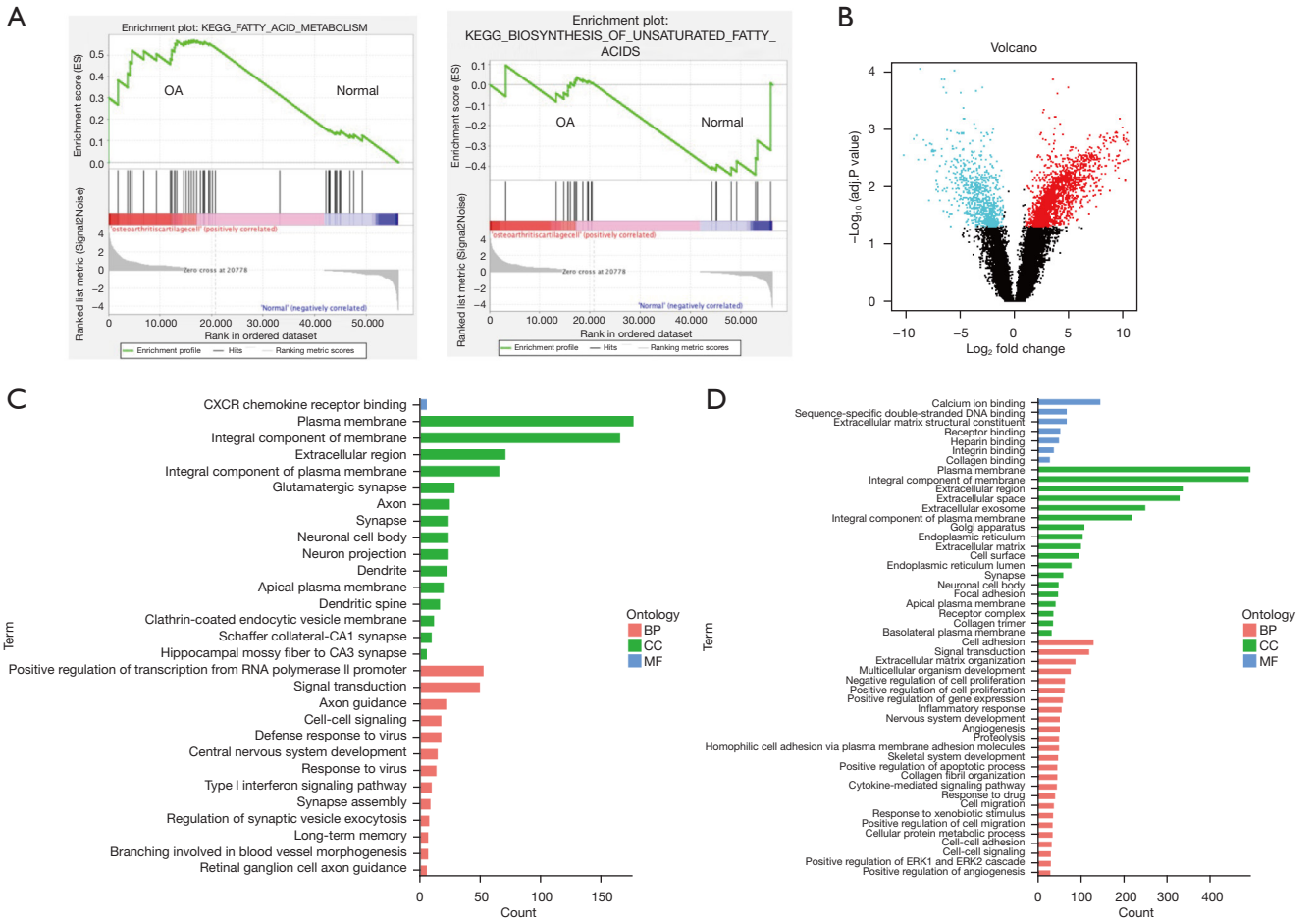

Figure 1 Unsaturated fatty acid metabolism is suppressed in osteoarthritic chondrocytes**.** (A) GSEA analysis indicates that fatty acid metabolism genes are significantly enriched in OA chondrocytes (left); in contrast, unsaturated fatty acid metabolism genes were suppressed (right). (B) The volcano plot analysis using RNAseq data of OA versus normal samples (n=3). The dots in blue (upper left quadrant) represent highly expressed genes in normal samples, while the dots in red denote highly expressed genes in the OA samples (fold change >2, P value <0.05). (C) Up-regulated GO terms from the RNAseq data (fold change >2, P value <0.05) of OA versus normal samples (n=3). (D) Down-regulated GO terms from the RNAseq data (fold change >2, P value <0.05) of OA versus normal samples (n=3). OA, osteoarthritis; BP, biological process; CC, cell component; MF, molecular function; GSEA, Gene Set Enrichment Analysis; GO, Gene Ontology.

osteoarthritic chondrocytes, and three osteoarthritic cartilage mesenchymal stromal cells. The RNAseq data of normal cartilage stromal cells and osteoarthritic chondrocytes were analyzed by GSEA. Interestingly, even though fatty acid metabolism was significantly enriched in osteoarthritic chondrocytes, unsaturated fatty acid metabolism was significantly inhibited (*Figure 1A*), which suggests that reduced unsaturated fatty acids might have a crucial role in OA progression. Next, we screened for genes that were significantly differentially expressed between osteoarthritic and normal chondrocytes (P<0.05, logFC >1) (*Figure 1B*) and performed a GO analysis for the up-regulated or down-regulated genes, respectively. A couple of the shared GO terms indicated that both up- and down-regulated genes were enriched in regulating the functions of cell membrane components (*Figure 1C*,*1D*). Thus, unsaturated fatty acid metabolism was inhibited in osteoarthritic chondrocytes.

SCD1 expression is reduced in OA articular cartilage

Since PUFA could not be made in vertebrate cell

lines, MUFA were synthesized to offset their PUFA deficiency (22). Based on the GSEA analysis, we hypothesized that MUFA biosynthesis was inhibited in OA. As SCD1 is an important rate-limiting enzyme in the anabolic process of MUFAs, the effect of SCD1 alterations in human OA articular cartilage was examined. When the cartilage specimens were stained with Safranin O/fast green and hematoxylin and eosin (HE) to determine the degree of deterioration, we found the superficial portion of normal articular cartilage was smooth, with just minor surface erosions. The superficial portion of OA cartilage, on the other hand, was cracked and fissured, with extensive fibrillation or cartilage erosion reaching down to the subchondral bone (*Figure 2A*). The Kellgren-Lawrence (K-L) OA stages were significantly higher than those of the controls (*Figure 2B*). Western blotting was used to compare SCD1 expression in OA cartilage versus normal cartilage (*Figure 2C*). SCD1 expression was found to be significantly lower in OA cartilage than in normal cartilage. We then performed surgical DMM on two-month-old wild-type C57BL6/J mice. As expected, the DMM operation significantly contributed to the progressive weakening and degradation of the articular cartilage compared with the sham surgery (*Figure 2D*). The maximal OARSI score of either articular surface showed that cartilage destruction was more severe in DMM mice (*Figure 2E*). Four weeks after DMM, the steady decrease of SCD1 protein in the articular cartilage was confirmed by Western blot assays (*Figure 2F*). As a result, we concluded that SCD1 expression is reduced in OA articular cartilage.

SCD1 deficiency in mouse chondrocytes accelerates cartilage degeneration after injury

To determine whether SCD1 regulates the expression of essential proteins associated with cartilage protection or injury, we generated and subjected the SCD1-KO mice to DMM. The efficiency of SCD1 knockdown in knee cartilage was verified by Western blot (*Figure 3A*). There were no significant abnormalities in the mice on joint X-ray examination at 6 months of age (Figure S1). In addition, by 6 months of age, SCD1-KO mice exhibited significantly increased expression of the chondrocyte hypertrophy marker, collagen type X (Col-X). In contrast, the expression of lubricin (a surface-active mucinous glycoprotein produced in the synovial joint that plays a crucial role in cartilage integrity) was significantly reduced in SCD1-KO mice compared with WT mice (*Figure 3B*). Quantification showed that SCD1-KO mice had a lower percentage of lubricin$^+$ chondrocytes and a higher percentage of Col-X$^+$ chondrocytes in both the upper and lower layers than wild-type mice (*Figure 3C*,*3D*). In addition, SCD1-KO mice had significantly more severe post-injury-induced cartilage damage and exhibited more severe signs of OA than WT mice at 4 and 8 weeks after DMM surgery (*Figure 3E*). The OARSI score of the tibia, femur or either articular surface confirmed that cartilage breakdown was more severe in SCD1-KO mice (*Figure 3F*). These histological changes in SCD1-KO animals reflected a transition from a homeostatic to a more degenerative state, as seen by the altered matrix and enhanced chondrocyte hypertrophy. Thus, these data suggested that SCD1 deficiency accelerated ECM degradation and the progression of OA after injury.

Ferroptosis in OA chondrocytes is regulated by SCD1 in vivo

The primary mechanism of ferroptosis is to induce lipid peroxidation of highly abundant PUFA on the cell membrane under the catalysis of ferrous iron or ester oxygenase. Previous GO analysis in osteoarthritic chondrocytes suggested that up- and down-regulated differentially expressed genes were enriched in the cell membrane component, leading us to speculate that chondrocytes may have undergone lipid peroxidation and ferroptosis in OA progression. To determine whether chondrocyte ferroptosis played a role in the progression of OA, we used immunofluorescence staining to look for the expression of glutathione peroxidase 4 (GPX4) (23), a crucial antioxidant enzyme and regulator of ferroptosis. The results showed that GPX4 expression was significantly lower in human OA cartilage than in normal tissues (*Figure 4A*,*4B*). In addition, P53 is another vital target protein for cell ferroptosis, which can potentiate ferroptosis by suppressing the transcription of system xc–subunit SLC7A11 (24). A significantly decreased expression of P53 was confirmed by immunohistochemical staining in the human OA cartilage specimens compared with the controls (*Figure 4C*,*4D*). To further verify the association between the deletion of SCD1 and ferroptosis in chondrocytes, we then looked at ferroptosis core regulator expression levels in mouse cartilage. The results showed that GPX4 expression was significantly down-regulated, and P53 expression was significantly elevated in the cartilage of SCD1-KO mice compared with WT mice 4 weeks after DMM surgery (*Figure 4E-4G*), which was similar to the human

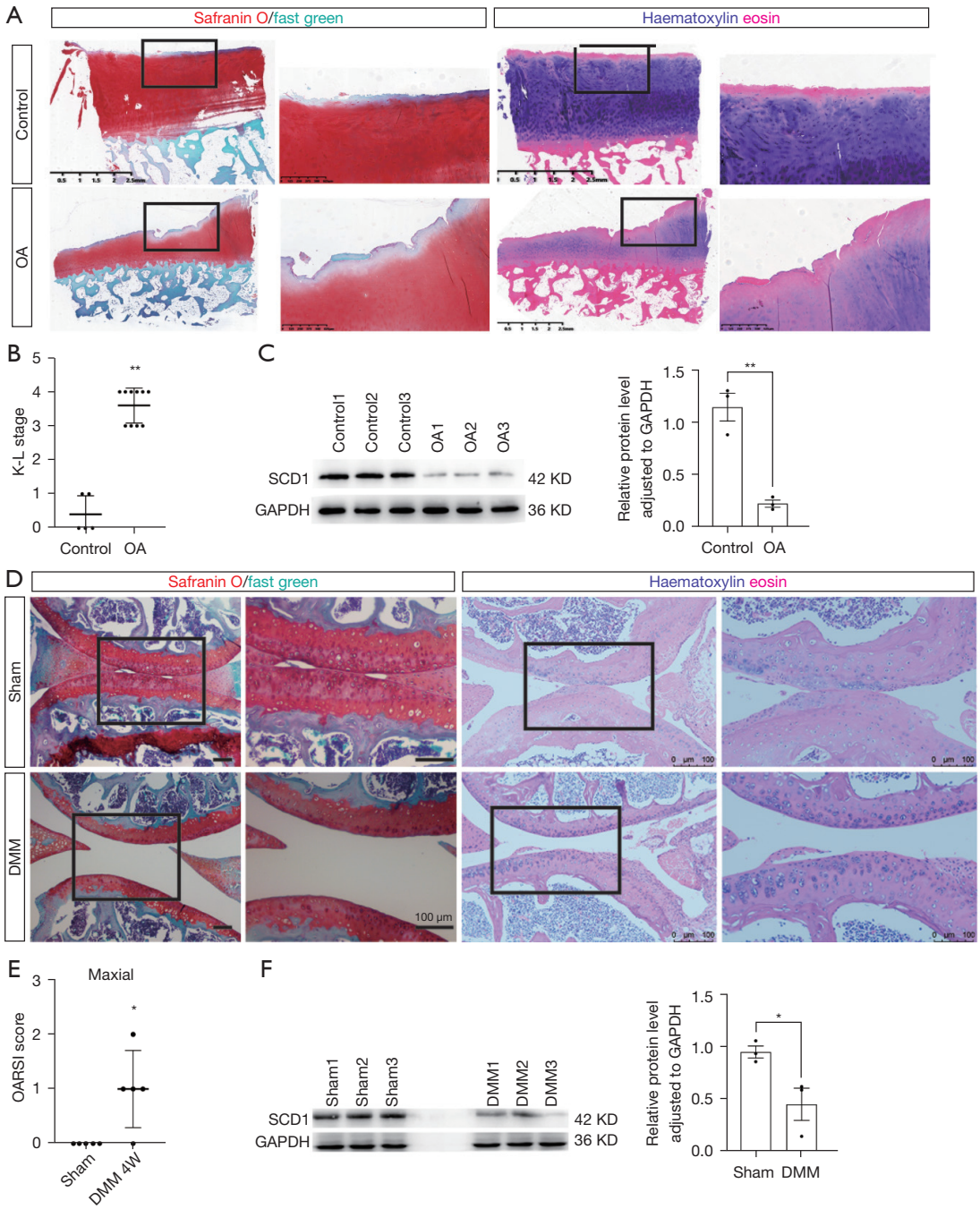

Figure 2 SCD1 expression is reduced in articular cartilage in OA. (A) The representative histopathological Safranin O/Fast Green (left) and hematoxylin and eosin (right) staining of cartilage tissues from normal humans (n=5) and patients with OA (n=10). Boxed areas are shown at higher magnification to the right. The same orientation is applied to all panels below. (B) The Kellgren-Lawrence grades of normal and OA cartilage. (C) Immunoblotting analysis of SCD1 protein levels in cartilage from normal humans and patients with OA. (D) The representative Safranin O/Fast Green (left) and hematoxylin and eosin (right) staining of knee sections in wild-type mice at 4 weeks after sham or DMM surgery. Scale bars: 100 μm. (E) OARSI scores of knee joint cartilage for wild-type mice at 4 weeks after sham or DMM surgery. Error bars: SD. n=5 mice, *, P<0.05; **, P<0.01, Mann-Whitney test. (F) Immunoblotting analysis of SCD1 protein levels in the cartilage of wild-type mice at 4 weeks after sham or DMM surgery. OA, osteoarthritis; SCD1, Stearoyl-CoA desaturase1; DMM, destabilization of medial meniscus; OARSI, the Osteoarthritis Research Society International.

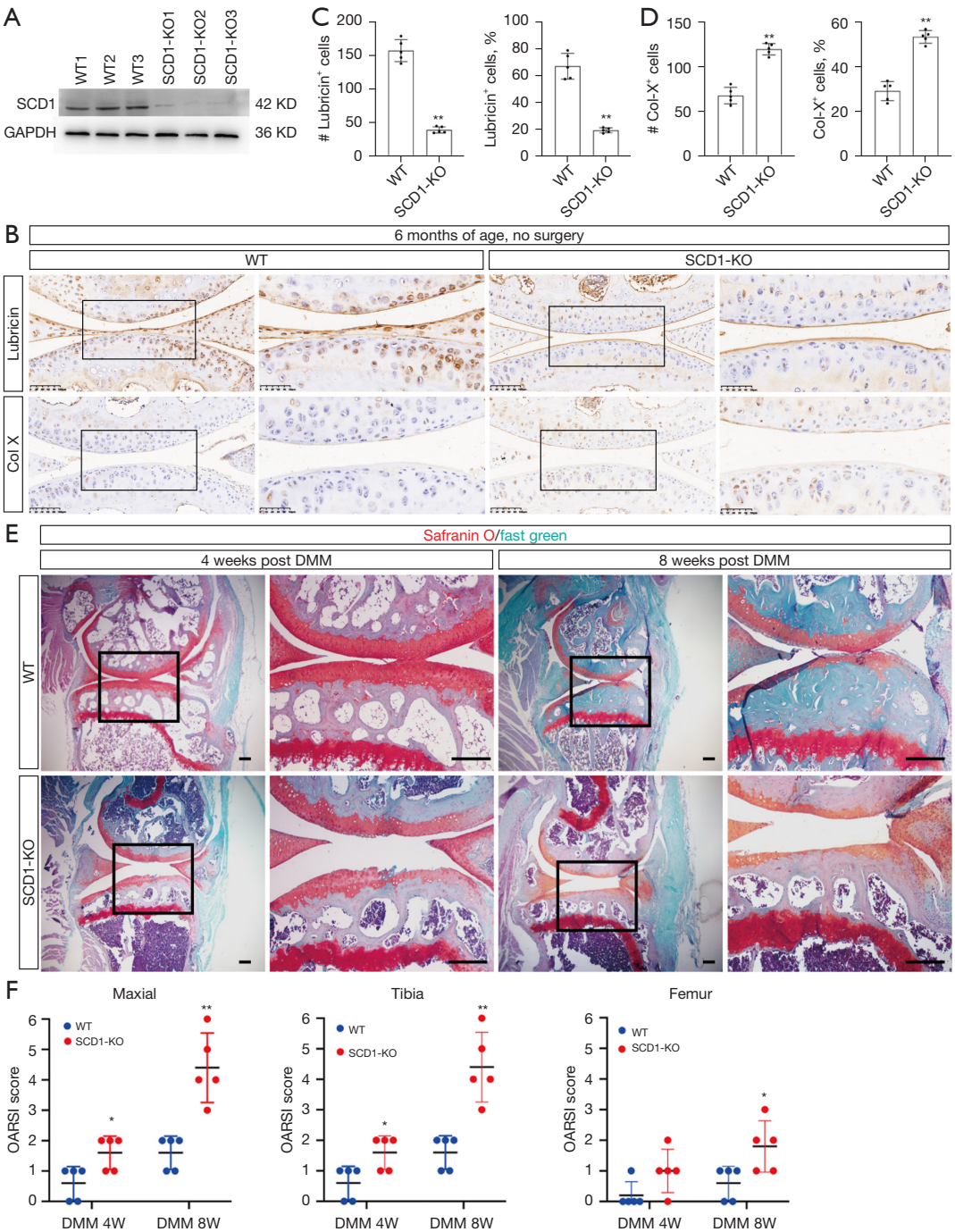

Figure 3 SCD1 deficiency accelerates cartilage degeneration after injury in mice. (A) Immunoblotting analysis of SCD1 protein levels in cartilage from WT and SCD1-KO mice. (B) Immunohistochemical staining of lubricin (upper) and COL-X (lower) in the joint cartilage of WT and SCD1-KO mice at 6 months of age. (C,D) Quantification of absolute number and percentage of lubricin+(c) and COL-X+(d) cells in the upper layers of tibial and femoral articular cartilage from WT and SCD1-KO mice at 6 months of age. Error bars: SD (n=5); **, P<0.01, Mann-Whitney test. (E) Safranin O/Fast Green staining of joints from WT and SCD1-KO mice at 4 or 8 weeks after DMM (n=5); Scale bars: 50 μm. (F) OARSI grades for the WT and SCD1-KO mouse joints at 4 and 8 weeks after DMM surgery (n=5), *, P<0.05, **, P<0.01, Mann–Whitney test. WT, wild type; SCD1-KO, Stearoyl-CoA desaturase1-Knockout; OARSI, Osteoarthritis Research Society International; DMM, destabilization of medial meniscus.

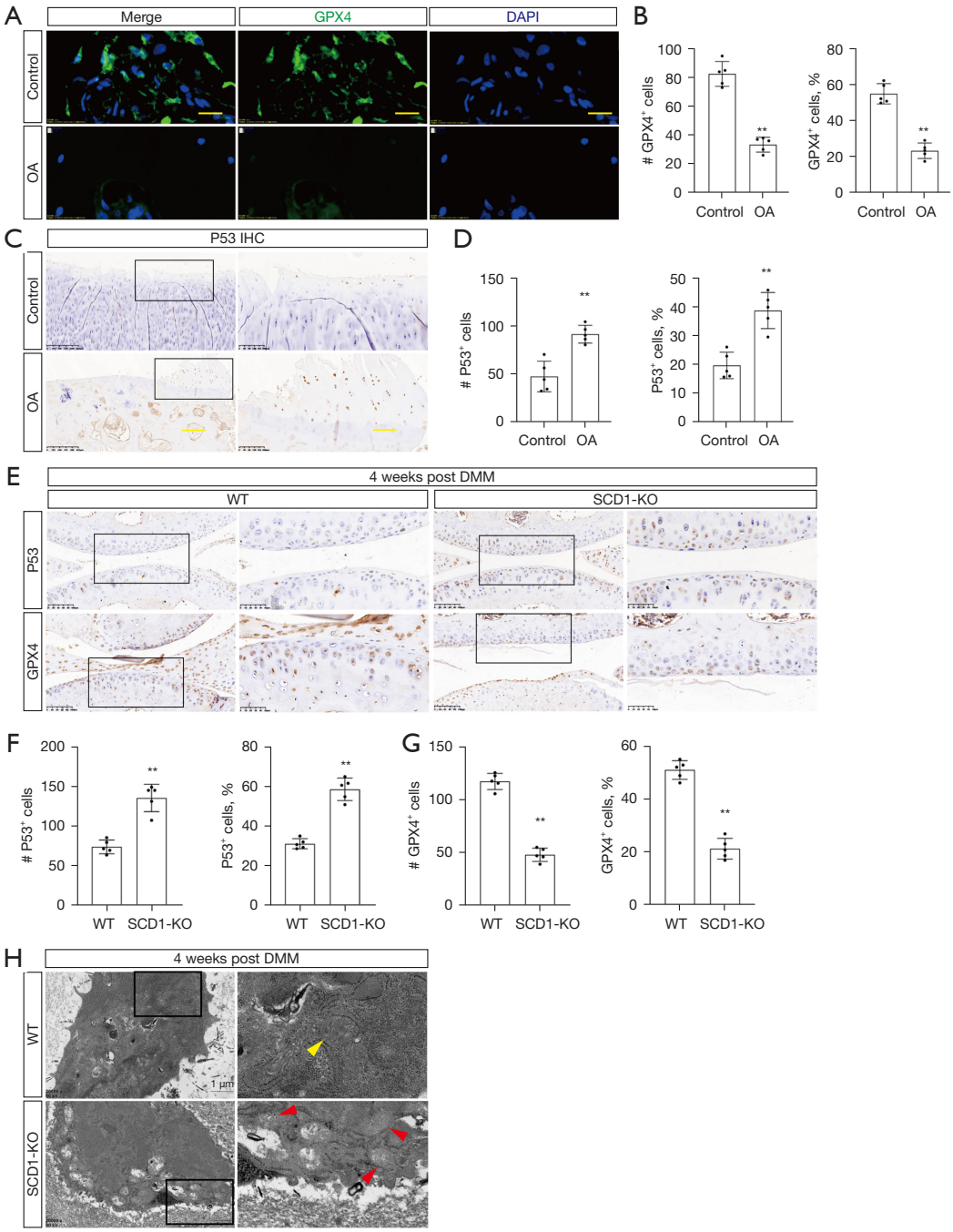

Figure 4 SCD1 regulates ferroptosis in osteoarthritic chondrocytes *in vivo*. (A,B) Immunofluorescence images (A) and quantification (B) of GPX4 in cartilage chondrocytes from normal humans and patients with OA (n=5), Scale bars: 10 μm. (C,D) Immunohistochemical staining (C) and quantification (D) of P53 in cartilage chondrocytes from normal humans and patients with OA (n=5), scale bars: 625 μm (L), 200 μm (R). (E-G) Immunohistochemical staining (E) and quantification (F,G) of P53 and GPX4 in dissected articular cartilage from WT and SCD1-KO mice at 4 weeks after DMM surgery (n=5), Scale bars: 100 μm (L) 50 μm (R). (H) Transmission electron microscopy shows characteristic changes in mitochondria within chondrocytes of WT and SCD1-KO mice at 4 weeks after DMM. Yellow arrow indicates normal mitochondria, and red arrows indicate mitochondria with reduced volume and increased bilayer density. (Data are expressed as mean ± SD. **, P<0.01). OA, osteoarthritis; IHC, immunohistochemistry; DMM, destabilization of medial meniscus; WT, wild type; SCD1-KO, Stearoyl-CoA desaturase1-Knockout.

specimen results. Furthermore, as was typical of ferroptosis, shrunken mitochondria were seen by transmission electron microscopy (TEM) in the cartilage from SCD1-KO mice 4 weeks after DMM surgery (*Figure 4H*). In summary, the findings suggested that the deletion of SCD1 exacerbated chondrocyte ferroptosis *in vivo*.

The SCD1-PPARG axis regulates chondrocyte ferroptosis

Peroxisome proliferator-activated receptors γ (PPARG) are ligand-activated nuclear receptors whose expression is directly regulated by long-chain fatty acids (LCFA) and SCD1 (25,26). PPARG deficiency in chondrocytes has previously been shown to lead to abnormal expression of OA-related metabolic and inflammatory genes (27,28). We hypothesized that PPARG was involved in the SCD1/ferroptosis regulatory pathway. To validate the importance of the SCD1-PPARG signaling axis in cartilage homeostasis regulation and determine if SCD1-PPARG could protect against OA-like pathologies in chondrocytes, we used immunohistochemistry to measure PPARG expression levels in SCD1-KO and WT mouse cartilage at 4 weeks after DMM surgery. The results suggested that PPARG expression was significantly lower in SCD1-KO than WT mouse cartilage (*Figure 5A*,*5B*). Furthermore, we constructed an OA model in ATDC5 chondrocytes stimulated with TNF-α, which are OA-associated pro-inflammatory cytokines (29). Significantly higher oxygen stress levels were detected by DHE assay (*Figure 5C*), and significantly increased apoptotic cells were detected by the apoptosis assay in the OA chondrocyte model compared with controls (*Figure 5D*). In addition, the expression of Mmp-13 mRNA was significantly increased in OA chondrocytes by qPCR analysis. In contrast, GPX4 and SCD1 gene expressions were significantly decreased, and these gene levels were consistent with the protein levels in our previous OA samples (*Figure 5E*). Next, we investigated the functions of SCD1 using siRNA knockdown in chondrocytes stimulated with TNF-α. Western blotting of SCD1, PPARG, and GPX4 protein expressions were performed and exhibited consistent trends (*Figure 5F*). The PPARG agonists reversed the reduction in PPARG and GPX4 in ATDC5 chondrocytes stimulated with siRNA (si-SCD1) (*Figure 5G*). Based on these findings, we propose that an SCD1-PPARG axis regulates ferroptosis in chondrocytes during OA progression.

Discussion

In this study, we found reduced SCD1 expression in human OA articular cartilage. Additionally, we produced conclusive genetic evidence that postnatal SCD1 deletion in cartilage causes OA-like diseases and accelerates OA progression after DMM surgery. Furthermore, we found that the SCD1-PPARG axis disrupts articular cartilage homeostasis by inducing catabolic and ferroptosis-related gene expression in ATDC5 chondrocytes. Consequently, our findings provide the first genetic evidence linking lower SCD1 expression to articular cartilage deterioration in OA.

It is suggested that metabolic variables play a role in the complex pathophysiology of OA, resulting in joint degradation and failure (21). In clinical practice, OA frequently co-occurs with metabolic illnesses and comorbidities, such as diabetes, hyperlipidemia, and cardiovascular disease (30). Loef *et al.* recently discovered a connection between high plasma postprandial SFA and PUFA levels and clinical hand and structural knee OA (31). Lu *et al.* found that total high fat and SFA diets were associated with greater structural knee OA progression, but MUFA might prevent radiographic advancement, which suggests that MUFA has a protective effect on OA progression (32). In the present study, we found significantly decreased SCD1 in the upper articular chondrocytes of human OA specimens. Although there was no indication of spontaneous OA in SCD1-KO mice up to 6 months old, we found a significant increase in cartilage degeneration markers among articular chondrocytes in the SCD1-KO mice, with increased COL-X expression and decreased lubricin expression. Moreover, significantly aggravated cartilage damage in the SCD1-KO mice was also observed after DMM-induced knee instability. The natural course of OA was established in this SCD1-KO mouse model, supporting the hypothesis that SCD1 deficiency aggravates cartilage degeneration and weakens regenerative ability after cartilage injury. As a result, decreased MUFA uptake likely impacts the health of articular chondrocytes, making them sensitive to OA risk factors in the elderly or those with injuries. Future studies on MUFA metabolism in joint cartilage and the outcome of OA induced by aging or damage are recommended.

Ferroptosis is a newly discovered mode of cell death in many tissues, including articular cartilage. The induction of ferroptosis is linked to cell death and

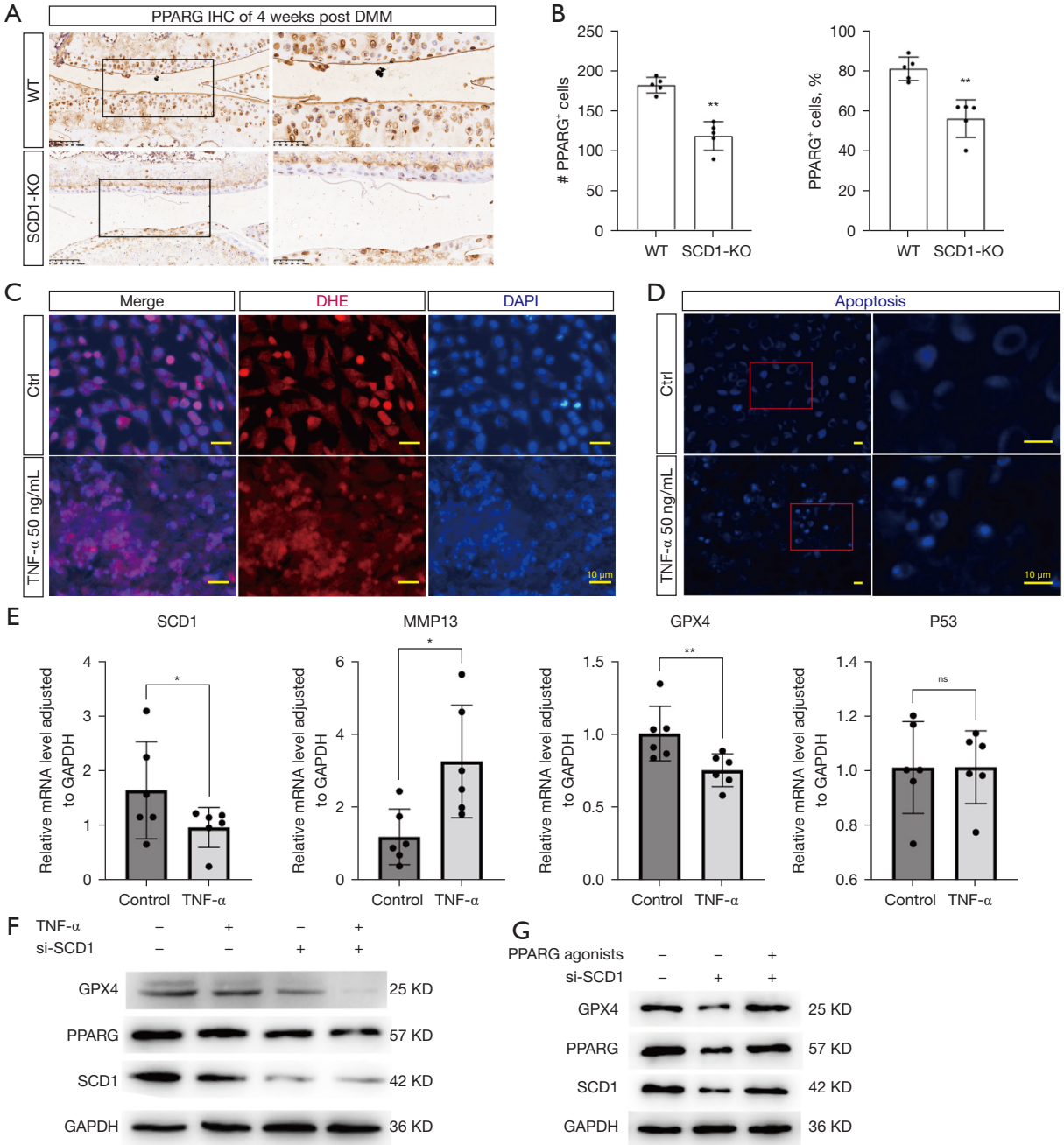

Figure 5 The SCD1-PPARG axis regulates chondrocyte ferroptosis. (A,B) Immunohistochemical staining (A) and quantification (B) of PPARG in dissected articular cartilage from WT and SCD1-KO mice 4 weeks after DMM surgery. Error bars: SD, (n=5), *, P<0.05; **, P<0.01; Mann–Whitney test; scale bars: 100 μm (L), 50 μm (R). (C) TNF-α (50 ng/mL) treatment for 2 hours to ATDC5 cells induces ROS activity (DHE staining), scale bars: 10 μm. (D) Apoptosis staining of ATDC5 cells after incubation with TNF-α (50 ng/mL) for 24 hours, scale bars: 10 μm. (E) Relative mRNA expression levels of SCD1, GPX4, MMP13, and P53 in ATDC5 cells after incubation with TNF-α (50 ng/mL) for 24 hours. Error bars: SD, (n=6), *, P<0.05, **, P<0.01, NS, not significant; Mann-Whitney test. (F) Immunoblotting analysis of SCD1, PPARG, GPX4, and GAPDH in ATDC5 chondrocytes after incubation with or without TNF-α and/or SCD1 for 24 hours (n=3). (G) Immunoblotting analysis of SCD1, PPARG, GPX4, and GAPDH in ATDC5 chondrocytes after incubation with or without siRNA (si-SCD1) and/or PPARG agonists for 24 hours (n=3). IHC, immunohistochemistry; DMM, destabilization of medial meniscus; WT, wild type; SCD1-KO, Stearoyl-CoA desaturase1-Knockout.

the pathophysiology of OA (33), and the inhibition of ferroptosis relieves the severity of experimentally induced OA (18). We showed that SCD1 deletion reduced the ferroptosis-related protein GPX4 and increased P53 protein *in vivo* and *in vitro*, respectively. However, there is no gold standard for detecting ferroptosis because the initial signals and molecular regulators of distinct forms of cell death are similar (12). In different diseases, GPX4 and P53 are both engaged in apoptosis and necrosis (34,35). In ATDC5 chondrocytes activated with TNF-α, we also found apoptosis and enhanced oxygen stress. In this study, we hypothesized that suppressing SCD1 changed the composition of membrane phospholipids, reducing the quantity of monounsaturated fatty acyl chains, increasing polyunsaturated fatty acyl chains, and decreasing membrane-localized antioxidants. This change in membrane composition increased the substrate for membrane lipid oxidation, making cells more vulnerable to ferroptosis inducers, which caused cell death by increasing lipid oxidation. Further studies of the regulatory networks that control ferroptosis via the SCD1 protein in the context of OA are warranted.

The precise mechanism for MUFA metabolism to maintain articular cartilage health is likely multi-faceted and requires additional investigation. Our findings show that cell survival and catabolic gene expression are both sensitive to changes in MUFA absorption. In addition to producing energy, MUFA can be nitrated to produce nitro-oleic acid. Nitro-oleic acid activates several signaling pathways, including those that have anti-inflammatory and antioxidant effects (36). Future research is needed to discover the specific metabolite pathways that connect poor MUFA metabolism to altered gene expression in OA articular cartilage.

There were several limitations in this study. First, this was an animal study with limited evidence for clinical practice. Second, the mechanism of SCD1 in regulating ferroptosis was not investigated. Thus, these results should be interpreted cautiously.

Conclusions

Deficiency of SCD1 is highly associated with OA by inducing ferroptosis in chondrocytes. The SCD1-PPARG axis is a potential target for the treatment of OA.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6630/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6630/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of Chongqing Medical University's University-Town Hospital (No. 2021.5.28/LL-202133) and informed consent was taken from all the patients. Animal experiments were performed under a project license (No. 2021.5.28/LL-202133) granted by the ethics board of the University-town Hospital of Chongqing Medical University, in compliance with national guidelines for the care and use of animals.

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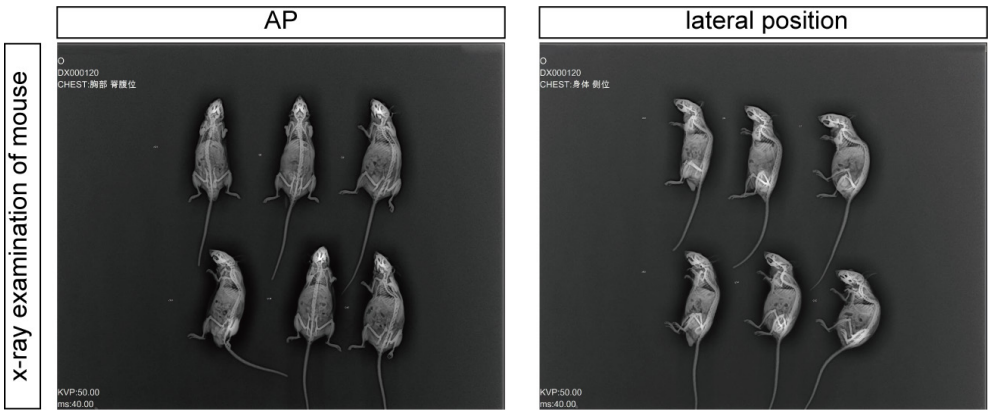

Figure S1 The joint x-ray examination from WT (upper) and SCD1-KO (lower) mice at 6 months of age.

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