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## Peer Review File

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### Reviewer A

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. In the manuscript “SCD1 deficiency exacerbates osteoarthritis by inducing ferroptosis in chondrocytes”, authors investigated the role of the SCD1 protein in the degenerative process of OA.

Couple questions are required to be answered before it will be accepted.

(1) Comment 1: What were the roles of ferroptosis in OA? Please state in the introduction.

Reply 1: Thank you for your advice, there is a close relationship between chondrocyte death and OA. Ferroptosis is a new type of cell death. Some studies have discovered chondrocytes Ferroptosis in OA. we added some text ....(see Page4, line 115-118)

Changes in the text: Because chondrocytes are the only cell type in articular cartilage, their survival is critical to maintaining the integrity of the cartilage. Ferroptosis in OA has been extensively studied. Increasing data suggests that chondrocyte ferroptosis is linked to the etiology of OA.

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(2) Comment 2: In the introduction, it was proposed to add related reference (DOI: 10.21037/atm-21-4617) about the biological functions of stearoyl-CoA desaturase (SCD1).

Reply 2: Thank you for your advice, in order to better explain the biological function of SCD1, we added the reference. (see Page4, line 102)

Changes in the text: we added the reference 7 and the order of other reference has been adjusted accordingly.

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(3) Comment 3: Why to focus on SCD1 in the study? Please state in the introduction.

Reply 3: Thank you for your advice, now many studies have found that there is a correlation between the proportion of eating unsaturated fatty acids and the incidence rate of OA. SCD1 is an important rate limiting enzyme in the production of unsaturated fatty acids. We speculate that the damaged function of SCD1 may affect the proportion of unsaturated fatty acids, thereby affecting OA. In addition, the change of the proportion of unsaturated fatty acids on the cell membrane may induce lipid peroxidation on the cell membrane and induce cell death. Therefore, we focus on SCD1. we added some text. (see Page4, line 109-111)

Changes in the text: So, might SCD1 affect chondrocyte destiny and thus the incidence and progression of OA via modulating lipid balance and cell membrane components?

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(4) Comment 4: In the methods, please state clearly the numbers of collected patients, and inclusion criteria.

Reply 4: Thank you for your advice, we added some text ....(see Page4, line 155-162)

Changes in the text: Human cartilage samples were collected from 10 patients who had total knee arthroplasty and 5 patients who had traumatic amputation. Inclusive criteria: 1. Age 50-70; 2. Patients with knee osteoarthritis, K-L grade 3 or above, who need total knee replacement;

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3. Or healthy patients who need amputation due to trauma. Exclusion criteria: 1. Age over 70 years old or less than 50 years old; 2. Arthritis patients with rheumatic, rheumatoid, gout, traumatic arthritis or other autoimmune diseases; 3. Have received arthroscopic surgery during the period; 4. Patients with serious medical diseases or chronic immune system diseases.

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(5) Comment 5: Please describe the SCD1-KO mice in detail in the methods.

Reply 5: Thank you for your advice, we added some SCD1-KO data ....(see Page5, line 166-178) (see Page6, line 188-190)

Changes in the text: 8-week-old male WT (C57BL/6J, n=40, Weight 18-22g) mice were provided by the Experimental Animal Center of Chongqing Medical University(Chongqing China).and 8-week-old male SCD1-KO (C57BL/6J, n=20, Weight 18-22g) 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 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.

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(6) Comment 6: Please state clearly the target sequence of siRNA.

Reply 6: Thank you for your advice, we added the nucleic acid sequences of siRNA and NC (Negative control) are shown in Table 2 .(see Page7, line 218-219, line 665-666Table2)

Changes in the text: The nucleic acid sequences of siRNA and NC (Negative control) are shown in Table 2.

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(7) Comment 7: Please perform statistical analysis about Western blot.

Reply 7: Thank you for your advice, we added statistical analysis about Western blot....(see Figure2-revised c,f)

Changes in the text: Figure2-revised (c,f)

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(8) Comment 8: It was better to further test using conditional knockout of SCD1 in chondrocytes.

Reply 8: Thank you for your advice. This is also our future research plan. As the New Year is approaching, all conditions are temporarily limited. Later, we will use chondrocyte conditional knockout SCD1 mice to carry out research on osteoarthritis.

Changes in the text: NA

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### **Reviewer B**

1. The article follows the ARRIVE checklist for reporting standards.

Reply: Thank you for your advice, we revise manuscript according to the attached checklist. we added some text ....(see Page5, line 166-179).

Changes in the text: 8-week-old male WT (C57BL/6J, n=40, Weight 18-22g) mice were provided by the Experimental Animal Center of Chongqing Medical University(Chongqing China).and 8-week-old male SCD1-KO (C57BL/6J, n=20, Weight 18-22g) 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.

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2. For any experiments involving animals, the authors must indicate the nature of the ethical review permissions, relevant licenses (e.g., Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals by which the research was conducted.

Reply: Thank you for your advice, we have modified Ethical statement in both the "Method" section of Main Text and the "Ethical Statement" section of Footnote as advised (see Page 5, line 152-155)" (see Page 15, line 472-479)"

Change in the text: Experiments were performed under a project license (No. 2021.5.28/LL-202133) granted by committee/ethics board of Chongqing Medical University's University-town Hospital, in compliance with China's national or institutional guidelines for the care and use of animals.

Change in the Footnote: Ethical Statement: Experiments were performed under a project license (No. 2021.5.28/LL-202133) granted by committee/ethics board of Chongqing Medical University's University-town Hospital, in compliance with China's national or institutional guidelines for the care and use of animals. This study was performed in line with the principles of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study. 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.

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3. Figure 3

Please explain DMM in the legend.

Reply: Thank you for your advise. we have modified our text as advised (see Page 22, line 637-638)"

Changes in the text: Destabilization of medial meniscus (DMM).

4. Figure 4

a) The scale bar is not clear, please provide it in the legend.

Reply a: Thank you for your advise. we have modified our text as advised (see Page 23, line 645-646)"

Changes in the text: Scale bars: 625  $\mu\text{m}$  (L), 200  $\mu\text{m}$ (R). (E,F,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  $\mu\text{m}$  (L), 50  $\mu\text{m}$ (R).

b) There is only one yellow arrow in the figure.

Reply b: Thank you for your advise. we have modified our text as advised (see Page 24, line 650)"

Changes in the text: Yellow arrow indicate normal mitochondria,

5. Figure 5

a) Figure 5C and 5D are not cited in the main text.

Reply a: Thank you for your advise. we have modified our text as advised (see Page 12, line 376-378)"

Changes in the text: 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).

b) The scale bar is not clear, please provide it in the legend.

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Reply: Thank you for your advise. we have modified our text as advised (see Page 24, line 660-661)"

Changes in the text: Mann–Whitney test; Scale bars: 100  $\mu\text{m}$  (L), 50  $\mu\text{m}$ (R).

c) Please check if the figure matches with the legend.

Reply c: Thank you for your advise. we have modified our Figure5 as advised (see Page 24, line 655-656 and Figure5-revised)"

Changes in the text: Figure5-revised

d) Please explain \*, \*\*, and ns in 5E's legend.

Reply c: Thank you for your advise. we have modified our Figure5 as advised (see Page 25, line 665-666 )"

Changes in the text: Error bars: SD, (n=6), \*,  $P<0.05$ , \*\*,  $P<0.01$ , NS, not significant; Mann–Whitney test.

## 6. Figure S1

Please provide figure S1's legend.

Reply: Thank you for your advise. we added figure s1's legend (see Page 25, line 674-676)"

Changes in the text: Figure s1: The joint x-ray examination from WT (upper) and SCD1-KO (lower) mice at 6 months of age.