

# Increased cholesterol uptake mediated by FABP4 induces OA chondrocyte phenotype through autophagy regulation

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**DISCLOSURE:** All authors have nothing to declare

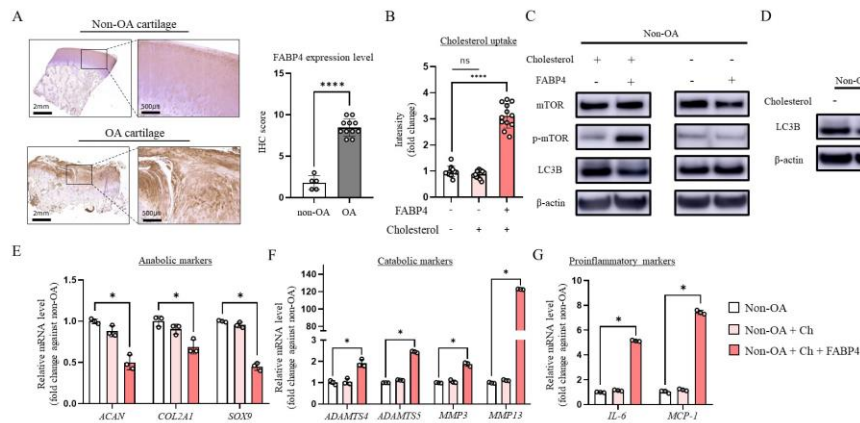
**INTRODUCTION:** Osteoarthritis (OA) is characterized by synovial fibrosis, subchondral bone changes and articular cartilage degradation, resulting in physical disability and discomfort. During OA pathogenesis, non-OA chondrocytes are shifted into OA chondrocytes which upregulate catabolic factors and downregulate anabolic factors, thereby degrading articular cartilage. Various factors contribute to this phenotypical shift, such as altered cholesterol metabolism. Fatty acid-binding protein 4 (FABP4) is involved in cholesterol efflux and influx in macrophage and is also increased in OA cartilage. However, the roles of FABP4 in OA chondrocytes has not been fully studied. Hence, we investigated the mechanism of inducing OA chondrocyte phenotypes through the regulation of FABP4-mediated cholesterol uptake.

**METHODS:** Human OA cartilage tissue were obtained from patients undergoing total knee replacement, and non-OA cartilage tissues were separated from the patients who underwent tumor removal in knee joint and were used as a control group. Non-OA and OA chondrocytes were isolated by digesting non-OA and OA cartilage, respectively. FABP4 expression in non-OA and OA chondrocytes and cartilage was evaluated using RT-PCR, western blot, and immunohistochemistry. Cholesterol uptake was assessed using NBD-tagged cholesterol. Autophagic change were investigated by western blot and immunofluorescence. Chondrocyte pellets were formed in chondrogenic media to investigate proteoglycan synthesis and anabolic/catabolic marker expression using histological staining and RT-PCR, respectively.

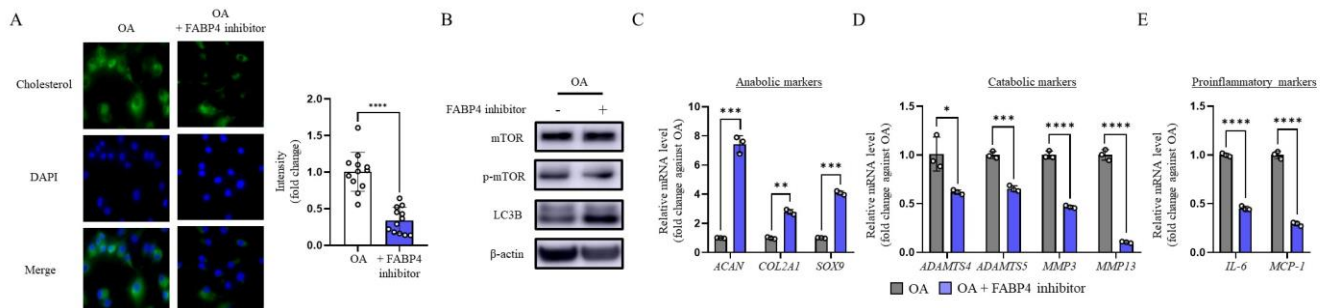
**RESULTS:** OA chondrocytes and cartilage has significantly upregulated FABP4 expression (Figure 1A). Cholesterol uptake was significantly enhanced in chondrocytes co-treated with cholesterol and FABP4 in comparison with chondrocytes treated with cholesterol only (Figure 1B). Increased cholesterol uptake through FABP4 declined autophagy signaling by increasing mTOR activity and reducing LC3B expression (Figure 1C). These results were cholesterol uptake dependent as any significant differences were not shown in the group treated with either FABP4 or cholesterol (Figure 1D). Non-OA chondrocytes co-treated with cholesterol and FABP4 had reduced anabolic factor expression while increasing catabolic and proinflammatory factors, indicating phenotypical alteration of non-OA chondrocytes to OA chondrocytes (Figure 1E, F and G). On the contrary, treatment with FABP4 inhibitor to OA chondrocytes decreased intracellular cholesterol levels and increased autophagic activity (Figure 2A and B). Furthermore, OA chondrocyte phenotypes were improved by FABP4 inhibitor treatment, leading to ameliorated anabolic and catabolic marker expression (Figure 2C, D and E).

**DISCUSSION:** Our results demonstrate that FABP4 increases cholesterol uptake in chondrocytes, which in turn negatively controls autophagy activity and induces OA phenotypes. Except for the role of cholesterol transporter, it has been reported that FABP4 could act as a signaling molecule by which knockdown of FABP4 activates PPAR $\gamma$  and thereby regulates NF- $\kappa$ B signaling pathway in IL-1 $\beta$ -induced chondrocytes. Further studies would be required to investigate the possible role of FABP4 as a signaling molecule in OA chondrocytes. Moreover, this study assessed the role of FABP4 in the chondrocytes *in vitro*, hence further *in vivo* animal study would be conducted to evaluate its potential as a therapeutic target for OA treatment.

**SIGNIFICANCE:** The present study identified another mechanism for cholesterol uptake by FABP4, which could lead to induction of OA chondrocyte phenotypes by regulating autophagy signaling.



**Figure 1.** FABP4 is upregulated in OA cartilage and FABP4-mediated cholesterol uptake reduces autophagy and induces OA phenotypes.



**Figure 2.** FABP4 inhibitor treatment reduces intracellular cholesterol of OA chondrocytes and improves autophagy and OA phenotypes.