Acetyl CoA carboxylase 1 (ACC1) mediated regulation of protein lysine malonylation and its role in osteoarthritis

Anupama Binoy[1], Huanhuan Liu[1], Anna Miller[1], Tom Martino[1], Siqi Ren[1], Shadi Moradi[1], and Shouan Zhu[*]
[1]Ohio Musculoskeletal & Neurological Institute, Ohio University, OH; *zhus1@ohio.edu

Introduction: The prevalence of osteoarthritis (OA) has doubled since the 19th century, coinciding with an aging population and growing obesity epidemic. One common mechanism that both aging and obesity cause diseases is through disrupting metabolism. However, it is still unknown how aging and obesity interact to impair cellular metabolism in cartilage tissue and promote OA. Previously, we reported that protein post-translational malonylation (MaK) is increased in obesity and is associated with dysregulated cellular metabolism in chondrocytes. We have exciting new data showing that MaK is also increased in cartilage during aging. Interestingly, the enzyme which produces the precursor for MaK, acetyl-CoA carboxylase 1 (ACC1), is significantly higher in both aging and obesity conditions. Hence, we hypothesize that increase of ACC1 during aging and obesity promotes MaK and metabolic dysfunctions of chondrocytes, which eventually leads to OA progression. The main objective of this study is to elucidate the role of ACC1 in regulating MaK and cellular metabolism in chondrocytes for OA development.

Methods: Human cartilage samples from young (29, 36, 45 years old) and old (61, 66, 74 years old) donors, as well as joint samples from obese and lean mice were used to immunohistochemically stain for malonyl-lysine (CST, #14942s) and ACC1 (CST, #4190s). Primary chondrocytes isolated from ~7-day-old juvenile WT or *ACC1-CartKO* mice articular cartilage were treated with or without a pan ACC inhibitor, ND-630 (5 nM for 48 h). Proteins from the cells were immunoblotted for malonyl-lysine. The cells (60,000 cells per well) were further used to measure both the oxygen consumption rate (OCR, mitochondrial respiration) and extracellular acidification rate (EACR, glycolytic metabolism) by a Seahorse XFe24 Extracellular Flux Analyzer. LC-MS based targeted metabolomic analysis was also conducted on the mice cartilage intraarticularly injected with either EtOH or ND-630 (3mg/Kg). Mice (WT or *ACC1-CartKO* mice) were fed either a low-fat diet (LFD) (10% kcal fat; D12450Ji) or high-fat diet (HFD) (60% kcal fat; D12492i) beginning at 6 weeks of age until 26 weeks of age. Body fat and glucose tolerance testing were also performed. Knee joints from WT and *ACC1-CartKO* mice were then processed for OA histopathology using standard methods and blinded semi-quantitative OARSI and Mankin scoring. Cu-catalysed alkyne azide cycloaddition reaction (CuAAC-click reaction) based chemoproteomic probes will be utilized to enrich malonylated protein from WT and *ACC1*$^{-/-}$ chondrocytes. The major hits among target proteins will be evaluated for their enzyme activity and cross talks in metabolic pathways.

Results: We first evaluated the aging and obesity associated increase in MaK and ACC1 expression in cartilage samples obtained from human and in joint samples from mice. Interestingly, expression of ACC1 as well as MaK was significantly higher in the cartilage samples from older donors in comparison to younger donors through immunohistochemical analysis (Fig 1A and C). Expression of ACC1 as well as MaK was also elevated in the joints from mice fed HFD in comparison to LFD (Fig 1B and D). The pharmacological inhibition of ACC by treating wild type (WT) primary chondrocytes with ND-630 effectively reduced the levels of MaK and malonyl-CoA *in vitro* (Fig 2A and B). Similar results were observed in primary chondrocytes extracted from ~7-day-old juvenile *ACC1-CartKO* mice when induced with 4-hydroxytamoxifen (10 µM for 48 h). ACC inhibition with ND-630 treatment downregulated basal mitochondrial respiration while upregulated glycolysis, resulting in an overall metabolic shift towards glycolytic phenotype in chondrocytes (Fig 2C, D and E). Consistently, LC-MS based targeted metabolomics revealed a distinct metabolic profile in the cartilage tissue with ACC1 inhibition. Interestingly, we also observed an increase in abundance of glycolytic metabolites like glucose-6-phosphate, fructose-6 -phosphate, and glycerol-6-phosphate (Fig 3A, B and C). *In vivo* experiments with ACC1 cartilage specific conditional knockout mice (Knee joint histopathology, metabolomics, Glucose tolerance test, NMR body composition and primary mechanical hyperalgesia) are ongoing in the lab. The click reaction based chemical probe to identify MaK proteins is also being synthesized and the corresponding experiments are ongoing in the lab.

Discussion: Our previous published results together with the current findings strongly suggest an important role of ACC1 in controlling chondrocyte cellular metabolism in aging and obesity conditions. The conventional role of ACC1 is to catalyze the first committed step towards fatty acid biosynthesis. In this project, we are investigating a novel role of ACC1 in regulating post-translational malonylation of a wide range of metabolic proteins. Results from this study could potentially uncover much broader functions of ACC1 in various metabolic pathways. Our ongoing effort to synthesize a click reaction-based alkyne tagged malonyl probe to enrich the malonylome in chondrocytes will help to identify the downstream metabolic targets of ACC1.

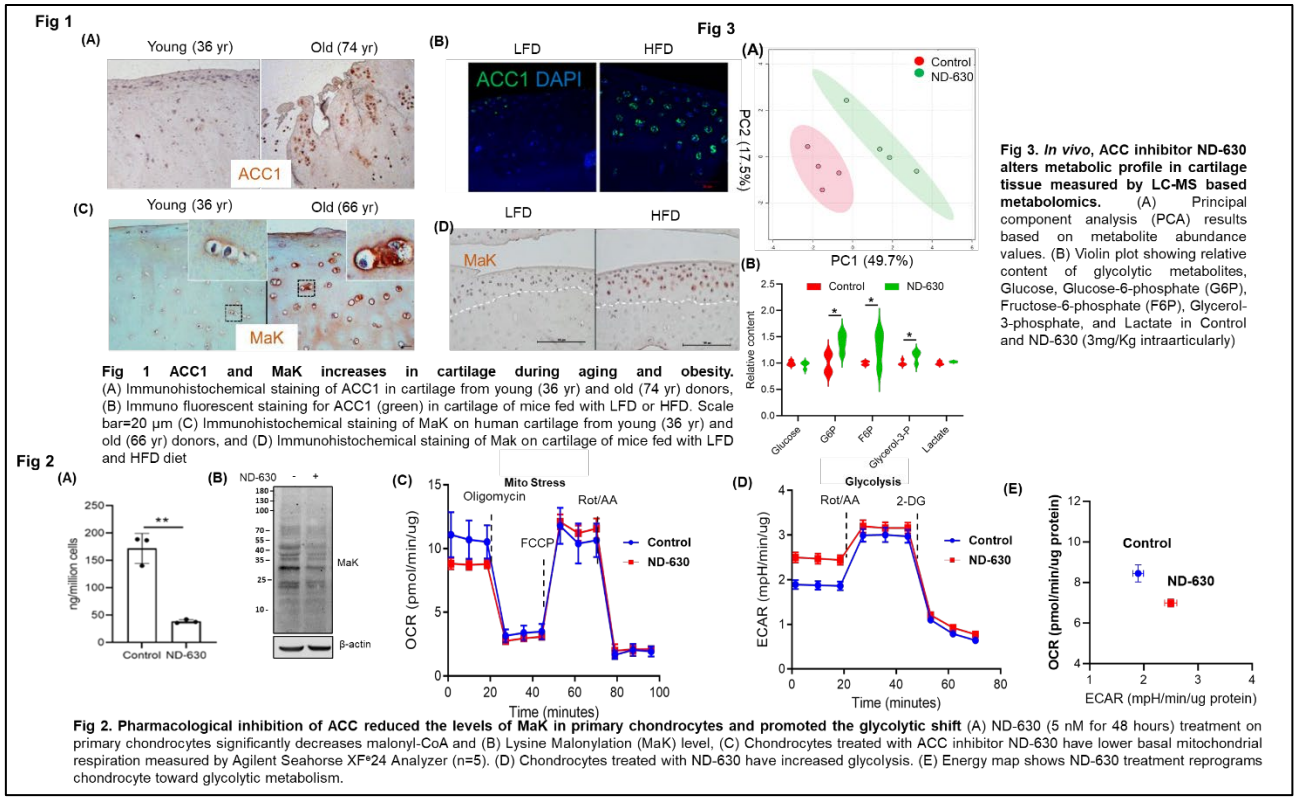

Fig 1 ACC1 and MaK increases in cartilage during aging and obesity. (A) Immunohistochemical staining of ACC1 in cartilage from young (36 yr) and old (74 yr) donors, (B) Immuno fluorescent staining for ACC1 (green) in cartilage of mice fed with LFD or HFD. Scale bar=20 µm (C) Immunohistochemical staining of MaK on human cartilage from young (36 yr) and old (66 yr) donors, and (D) Immunohistochemical staining of Mak on cartilage of mice fed with LFD and HFD diet

Fig 3. *In vivo*, ACC inhibitor ND-630 alters metabolic profile in cartilage tissue measured by LC-MS based metabolomics. (A) Principal component analysis (PCA) results based on metabolite abundance values. (B) Violin plot showing relative content of glycolytic metabolites, Glucose, Glucose-6-phosphate (G6P), Fructose-6-phosphate (F6P), Glycerol-3-phosphate, and Lactate in Control and ND-630 (3mg/Kg intraarticularly)

Fig 2. Pharmacological inhibition of ACC reduced the levels of MaK in primary chondrocytes and promoted the glycolytic shift (A) ND-630 (5 nM for 48 hours) treatment on primary chondrocytes significantly decreases malonyl-CoA and (B) Lysine Malonylation (MaK) level, (C) Chondrocytes treated with ACC inhibitor ND-630 have lower basal mitochondrial respiration measured by Agilent Seahorse XFe24 Analyzer (n=5). (D) Chondrocytes treated with ND-630 have increased glycolysis. (E) Energy map shows ND-630 treatment reprograms chondrocyte toward glycolytic metabolism.