Role of ACC1 in rewiring the chondrocyte metabolism during obesity and injury associated Osteoarthritis

Anupama Binoy[1#], Shadi Moradi[1], Huanhuan Liu[1], Abhijit Sukul[1], Siqi Ren[1], Anna Miller[1], and Shouan Zhu[*]

[1]Ohio Musculoskeletal & Neurological Institute, Ohio University, OH; [#]anupama@ohio.edu, *zhus1@ohio.edu

Introduction: Aging and obesity are the most common risk factors for Osteoarthritis (OA). 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. Interestingly, the enzyme that produces the precursor for MaK, acetyl-CoA carboxylase 1 (ACC1), is significantly higher in both aging and obesity conditions. Therefore, we hypothesize that increase in ACC1 during aging and obesity promotes MaK in cartilage and hence has a pivotal role in molecular level alteration, eventually leading to OA progression. The main objective of this study is to elucidate the role of ACC1 mediated upregulation of MaK during the progression of OA.

Methods: *ACC1-CKO* (*Aggrecan-Cre^{ERT2}:ACC1$^{flox/flox}$*) mice were generated by crossing *Aggrecan-Cre^{ERT2}* mice with WT mice (ACC1$^{flox/flox}$) and were injected with tamoxifen (100 mg/Kg of body weight or 150 µL from 15mg/ml stock prepared in peanut oil solution) for 5 consecutive days at P45 to knockout ACC1 gene in cartilage. One cohort of mice containing WT and *ACC1-CKO* (both male and female) were fed on low fat diet (LFD-10% fat) and high fat diet (HFD-60% fat) for 25 weeks to study the effect of ACC1 gene disruption on obesity induced OA. Weekly weight measurement, glucose tolerance test and knee hyperalgesia were performed on this cohort of mice. Another cohort of mice containing WT and *ACC1-CKO* (both male and female) was set for studying the effect on post-traumatic OA (PTOA), wherein a non-invasive anterior cruciate ligament rupture (ACLR) was performed on the left leg of the mice. Knee hyperalgesia measurement was performed for both ruptured and contralateral (CTRL) leg on 0th, 3rd, 7th, 14th, 21st till 56th day. Mice from both the cohort were sacrificed at the end of the experiment and OA histopathology followed by blinded semi-quantitative OARSI and Mankin scoring were performed using standard methods. The articular cartilage (femur condyles and tibial plateau) from both WT and *ACC1-CKO* mice were subjected to targeted metabolomics (carbon and amino acids metabolism) using a UHPLC system coupled with MS. An MaK antibody-based enrichment followed by label free quantification of malonylated proteins in *ACC1-CKO* and WT primary chondrocytes (isolated from 28 days old mice) was also performed to analyze the fold change difference in malonylation of proteins between *ACC1-CKO* and WT group. Sea horse experiment (using Seahorse XFe24 Extracellular Flux Analyzer) was performed to measure both the oxygen consumption rate (OCR, mitochondrial respiration) and extracellular acidification rate (EACR, glycolytic metabolism) on the 4-hydroxy tamoxifen treated primary chondrocytes isolated from ~7-day-old juvenile WT or *ACC1-CKO*.

Statistical methods: The OARSI and MANKIN scoring were plotted as mean ± SEM and evaluated by two-way ANOVA followed by Tukey's multiple comparison test. Graphs for Glycolytic Rate Assay and Mito Stress Assay were plotted as mean ± SEM. The graph of GAPDH activity was plotted as mean ± SEM and evaluated by student's t-test. The statistical significance was represented as 'ns' (non-significant), for $p > 0.05$ as *, for $p < 0.05$ as **, for $p < 0.001$ as *** and **** for $p < 0.0001$.

Results: We observed significantly higher expression of ACC1 as well as MaK in the cartilage during both aging and obesity. Weekly body weight measurement, Glucose tolerance test, and NMR body composition analysis of the mice fed on HFD and LFD revealed that cartilage specific genetic knockdown of ACC1 did not induce metabolic changes between WT and *ACC1-CKO* mice (data not shown). Histopathological analysis followed by OARSI and Mankin scoring of the knee joints from the same cohort of mice revealed that *ACC1-CKO* mice (both male and female) exhibited decreased cartilage damage and limited loss of proteoglycan staining even in HFD group (Fig 1A and B). *ACC1-CKO* mice subjected to non-invasive ACLR showed protection against the development of PTOA compared to WT controls, as demonstrated by OA histopathology and confirmed through blinded semi-quantitative OARSI and Mankin scoring (Fig 1C and D). Targeted metabolomic analyses of knee joints of adult *ACC1-CKO* and WT mice identified 376 metabolites out of which 26 metabolites were significantly upregulated and 16 metabolites were significantly downregulated (fold difference = 1 and p value <0.05). Indeed, pathway analysis showed that *ACC1-CKO* primarily altered metabolites associated with glycolytic, pentose phosphate, TCA cycle intermediates. Amino acids including branched-chain amino acids and aromatic amino acids were also observed to be accumulated in *ACC1-CKO* group (Fig 2A). Metabolites associated with purine and pyrimidine as well as glutathione metabolism were also seen to be altered due to *ACC1* disruption. Label free quantification of anti-MaK antibody enriched proteins revealed that there was decrease in malonylation of proteins with ACC1 disruption in primary chondrocytes. KEGG based analysis revealed that ACC1 knockdown resulted in decrease in malonylation of metabolic enzymes belonging to glycolysis/gluconeogenesis, biosynthesis of amino acids, pentose phosphate pathway, TCA cycle, pyruvate and aromatic amino acid metabolism (Fig 2A and B). ACC1 knockdown also downregulated basal mitochondrial respiration while upregulated glycolysis, in an overall metabolic shift towards glycolytic phenotype in chondrocytes (Fig 3A and B). ACC1 knockdown also resulted in increased GAPDH enzyme activity which substantiates the decrease in malonylation of GAPDH.

Discussion: Increased expression of ACC1 and therefore increased levels of post-translational malonylation of proteins in aging and obese conditions can lead to changes in the enzymatic activity of many metabolic enzymes which can be crucial for the overall maintenance of extracellular matrix homeostasis in the cartilage. Cartilage specific knockdown of ACC1 could protect mouse knee joints from developing obesity induced and PTOA. Accumulation of glycolytic and TCA cycle metabolites suggest ACC1 knockdown induced a metabolic reprogramming leading to glycolytic shift suggesting that ACC1 is an important mediator of chondrocyte metabolism. Moreover, the decreased malonylation of key glycolytic, and pentose phosphate pathway enzyme suggest for their increased enzymatic activity. Altogether, these results suggest that ACC1 plays an important role in regulating chondrocyte metabolism and OA development.

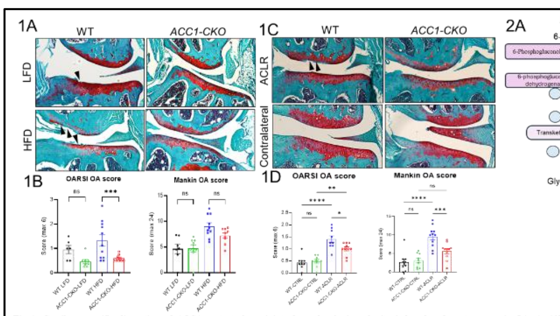

Fig 1: Cartilage specific disruption of ACC1 protects knee joints from developing obesity induced and post traumatic OA. A. 10X magnification images of Fast Green/Safranin Orange staining on the knee joints of female *ACC1-CKO* and WT mouse fed on LFD and HFD diet (n=6 for the WT-LFD, n=12 for the WT-HFD, n=11 for the *ACC1-CKO* LFD, and n=10 for the *ACC1-CKO* HFD, Males-not shown (n=14 for the WT-LFD, n=8 for the WT-HFD, n=8 for the *ACC1-CKO* LFD, and n=13 for the *ACC1-CKO* HFD) .B. OARSI and Mankin scoring. C. 10X magnification images of Fast Green/Safranin Orange staining on the ACLR and contralateral knee joints of female *ACC1-CKO* and WT mouse diet (n=7 for the WT-CTRL, n=9 for the WT-ACLR, n=8 for the *ACC1-CKO* CTRL, and n=10 for the *ACC1-CKO* ACLR, Males-not shown (n=9 for the WT-CTRL, n=7 for the WT-ACLR, n=11 for the *ACC1-CKO* CTRL, and n=12 for the *ACC1-CKO* ACLR). D. OARSI and Mankin scoring

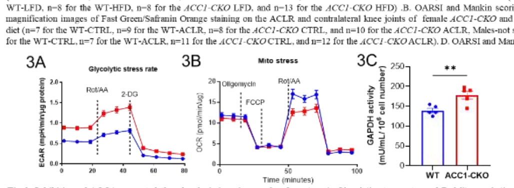

Fig 3: Inhibition of ACC1 promoted the glycolysis in primary chondrocytes. A. Glycolytic stress rate and B. Mito respiration stress rate measured on the primary chondrocytes isolated from ~7-day-old juvenile WT or ACC1 CKO mice (n=10). C. GAPDH enzyme activity assay (n=4)

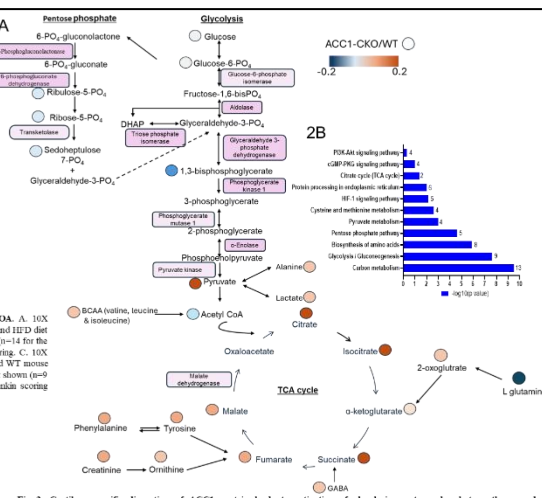

Fig 2: Cartilage specific disruption of ACC1 protein leads to activation of glycolysis, pentose phosphate pathway and accumulation of TCA cycle intermediate and amino acids: A. Overview of the metabolic map (based on KEGG database) of the majority of detected metabolites in in the knee joints of ACC1 CKO/WT mice (n=4 in each group). B. KEGG pathway enrichment analysis of proteins which were 2-fold less malonylated in ACC1 CKO than the WT primary chondrocytes. Numbers in the brackets show the number of malonylated proteins in the pathways.

Significance. In this project, we are investigating a novel role of ACC1 in regulating protein post-translational malonylation. Results from this study could potentially uncover much broader functions of ACC1 in various metabolic pathways and provide new therapeutic targets for OA.