Title: Role of Autophagy in Hepatic Proteome and its Post-translational Modification

Kamal Baral, Leah Spade, Arissa Mercer , Sadisha Shrestha S, Arya A Chandrasekhar, Gang Liu, Xiao-Ming Yin, and Bilon Khambu

Department of Pathology and Laboratory Medicine, School of Medicine, Tulane University

Introduction: Post translational modification (PTM) of the proteome via acetylation results in the homeostatic maintenance of the hepatic acetylome, the level of which reflects cellular metabolic state. The hepatic acetylome is involved in variety of hepatocellular processes including metabolic pathways, epigenetic modification, signal transduction, cell proliferation, and apoptosis. How liver maintains the homeostatic level of acetylome is unknown. Here we show that autophagy function is necessary to maintain the acetylation of hepatic proteome and hence maintain the acetylome by a non-degradative transcriptional pathway.

Methods: In this study various autophagy deficient mouse models were used to dissect the role of autophagy in hepatic acetylome regulation. Mice with hepatic deletion of autophagy-related gene 7 (*Atg7*) or autophagy-related gene 5 (*Atg5*) were bred crossing *Atg7* or *Atg5* floxed mice with *Alb-Cre* mice. Mice of both genders at the age of 9 weeks old were studied. Wild type (WT) mice were injected with chloroquine (60 mg/Kg, *i.p*) for 6 consecutive days to inhibit autophagy. Contrary to this, autophagy activation was done by fasting, rapamycin treatment or by genetic activation of autophagy ($Becn1^{-F121A/F121A}$). Total liver lysate and subcellular fraction autophagy impaired mice were analyzed by immunoblotting for acetylated lysine and multiple PTM. Liver sections stained for Acetyl-Lysine and Hoechst. Hepatic estimation of Acetyl-CoA, CoA and Histone H_3 acetylation, and quantitative PCR of various enzymes involved in acetyl-CoA regulation were analyzed. For rescue experiment, Acetyl-CoA was administered intraperitoneally (10mg/kg body weight) to $Atg5^{FF}$ and $Atg5^{-/-}$ mice for eight consecutive days. Liver sections were subjected to H&E staining and serum ALT was examined as a measure of liver injury. Human liver autopsy samples of chronic liver diseases were immunostained with acetyl lysine to validate the preclinical findings.

Results: Examination of PTM of hepatic proteome showed that ubiquitination, SUMOylation, methylation, ADP-ribosylation, and phosphorylation of hepatic proteome was significantly upregulated in autophagy-deficient liver. In contrast, the acetylation of hepatic proteome(acetylome) was dramatically downregulated in both autophagy-deficient and autophagy-defective livers. Cellar fractionation studies showed that the overall hepatic acetylome

covering nuclear, cytosolic, mitochondrial, membrane fractions were downregulated in autophagy-deficient liver. Contrary to this, autophagy activation by fasting, rapamycin treatment or by genetic activation of autophagy (Becn1$^{-F121A/F121A}$) increased the level of hepatic acetylome suggesting the critical role of autophagy in maintenance of hepatic acetylome. Furthermore, mechanistic studies showed that autophagy maintains levels of acetyl-CoA, an important intermediate metabolite required for protein acetylation. By downregulating key enzymes involved in the acetyl-CoA biosynthesis, such as *Acly, AceCS1, AceCS2, Mlycd*, and *Pdha1*, autophagy impairment significantly reduced hepatic acetyl-CoA production. Notably, in the autophagy-deficient liver, replenishing hepatic acetyl-CoA rescued the lower acetylome and protected against liver injury. Moreover, screening for transcription factors that regulate acetyl-CoA biosynthesis enzymes revealed multiple hits, including nuclear erythroid-derived 2-like 2 (*Nrf2*). The co-deletion of Nrf2 transcription factor rescued the expression of acetyl-CoA biosynthesis enzymes and rescued the lower hepatic acetylome levels. Finally, lower hepatic acetylome, autophagy defect and Nrf2 activation was observed in the human liver autopsy samples of chronic liver diseases validating the preclinical findings and suggesting the clinical relevance of the study.

Conclusion: Autophagy regulates hepatic acetylome through transcriptional regulation of the enzymes that synthesize acetyl-CoA.

Key words*: Hepatic proteome, hepatic Acetylome, autophagy, acetyl coenzyme-A*