Hardy Hang
L2
LSU Health Sciences Center, New Orleans, LA

Martin J. Ronis, PhD
LSUHSC, Department of Pharmacology and Experimental Therapeutics,
Comprehensive Alcohol Research Center

"Alcohol metabolism negatively affects early ATDC5 chondrocyte differentiation independent of *Nox4* expression"

Alcohol (ethanol) abuse is a widely recognized risk factor in the development of several diseases. Previous studies in our lab have shown that ethanol induces reactive oxygen species (ROS) production in osteogenic cells, which results in oxidative damage, skeletal dysfunction, and osteoporosis. Moreover, ethanol inhibits epiphyseal plate proliferation in longitudinal bones and affects chondrocyte function, which results in shorter bones. In osteogenic cells, a major source of ethanol-mediated ROS is derived from NADPH oxidases (NOXs). However, it remains uncertain if ROS produced by NOXs during alcohol metabolism result in impaired chondrocyte function and differentiation. In this study, we investigated if chronic alcohol exposure in chondrocytes will affect chondrogenesis through the induction of *Nox* expression and subsequent ROS production. To examine this hypothesis, the ATDC5 murine chondrogenic cell line was used to evaluate if physiologically relevant levels of ethanol and acetate, one of the products of alcohol metabolism, contribute to the dysregulation of chondrogenesis. In these preliminary studies, ATDC5 cells were grown to confluence and induced to differentiate for 7 or 14 days with vehicle controls, 50 mM ethanol, or 5 mM acetate. After differentiation, cells were stained with Alcian blue to evaluate cartilage formation. In addition, total RNA and subcellular protein fractions were isolated to perform RT-qPCR and Western blotting analyses to assess the regulation of several chondrocyte differentiation markers and NOX4 induction, respectively. Alcian blue staining demonstrates decreased cartilage formation with ethanol and acetate treatments ($p < 0.001$), and gene expression analyses similarly show that both treatments decrease expression of the chondrogenesis markers, *Acan*, *Col2a1*, and *Ihh* ($p < 0.01$), in early 7-day chondrocytes. Conversely, Alcian blue staining shows increased cartilage deposition with chronic ethanol treatment whereas acetate treatment does not significantly change deposition compared to the vehicle control in 14-day chondrocyte cultures. In addition, gene expression results show that that ethanol and acetate significantly downregulate *Col2a1* gene expression ($p < 0.001$) but do not affect expression of other chondrogenesis markers. In both early and mature chondrocyte cultures, *Nox4* mRNA was not significantly altered with either ethanol or acetate treatment. Overall, these results indicate that ethanol and acetate cause differential impairments in chondrocyte differentiation and function in a time-dependent manner independent of *Nox4* expression. Future analyses will explore the effects of oral ethanol and acetate administration on epiphyseal plate morphology and costal chondrocyte gene/protein expression in an acute binge mouse model. Supported in part by R37 AA018282 (M.J.R.).