Metabolic reprogramming and lipid distribution protects Gclm KO mice from alcohol induced steatosis

Srujana Golla\textsuperscript{1}, Kristopher W. Krausz\textsuperscript{1}, Vasilis Vasiiliou\textsuperscript{2} and Frank J. Gonzalez\textsuperscript{1}

\textsuperscript{1}Laboratory of Metabolism, Center for Cancer Research, NCI, NIH, Bethesda, MD 20892
\textsuperscript{2}Department of Environmental Health Sciences, Yale School of Public Health, New Haven, CT 06520

Alcoholism is an increasing global health problem and a significant proportion of alcohol-related deaths are due to liver failure are associated with chronic alcohol consumption. Alcoholic liver disease (ALD) is characterized by abnormal lipid accumulation called steatosis that progresses to steatohepatitis and can further lead to cirrhosis and liver failure. The liver damage caused by alcoholic steatosis is reversible with timely intervention that slows disease progression and fatal consequences. Chronic alcohol consumption influences the liver sensitivity and creates shift in redox balance that leads to impairment of fatty acid oxidation and promotes lipid accumulation. Hence a mechanistic understanding of redox homeostasis genes clarifies their specific role in ALD pathogenesis. In this study, a \textit{gamma-glutamylcysteine synthetase} (\textit{Gclm})-null mouse model was used to delineate the crucial role of glutathione and the underlying molecular mechanism of ALD using robust, state-of-the-art metabolomics and targeted lipidomics analysis. Significantly, the study aimed to focus on the global metabolic changes in the liver, role of lipid metabolism and effect of lipid distribution patterns in \textit{Gclm}-null mice fed with and without alcohol. Chronic alcohol treatment of \textit{Gclm}-null mice did not increase total body weight and/or fat accumulation in the liver. Principal components analysis of the liver metabolic fingerprints of alcohol exposure of the \textit{Gclm}-null and wild-type mice was found to be markedly different from that of non-alcohol treated mice (Figure 1). Comparison of the metabolic signatures through supervised orthogonal projection to latent structures analysis revealed significantly contributed metabolic signature of alcohol exposure in wild-type and \textit{Gclm}-null mice. Metabolomics analysis showed that not only total GSH levels but also the ratio of GSH/GSSG was significantly lower in alcohol-treated \textit{Gclm}-null mice compared to wild-type. Depleted GSH/GSSG would result in a shift of redox balance towards more oxidative state through the activation of antioxidant machinery. This adaptive response redirects the metabolic reprogramming of acetyl-CoA that prevents de novo fatty acid synthesis. Hence, lack of reducing equivalent in \textit{Gclm}-null mice protects against steatosis by impairment of de novo fatty acid synthesis, and redistribution of
metabolic flux into alternative pathways. Mass spectrometry coupled with targeted lipidomics analysis identified a total of 120 significant lipid species considering genotype and chronic alcohol exposure. The Triglyceride content is significantly rich in alcohol treated Gclm-null mice with increased unsaturation of fatty acid content ranging from 16:0/16:0/18:1 to 60:11 containing 1 to 11 carbon double bonds (Figure 2). Increase in the polyunsaturated fatty acids is known to decrease inflammation. The increased unsaturation in the triglycerides might be due to the shift of redox balance towards more oxidative state in Gclm-null mice due to impairment of GSH synthesis. This study demonstrates the biochemical adaptation and lipid metabolism regulation associated steatosis and ALD pathogenesis. The proficiency and integration of metabolomics and lipidomics enlightens the systems level understanding of the disease phenotype such as ALD.

**Figure 1**: Principal components analysis scatter plots (A) scores scatter plot of global metabolomic signature associated with alcohol exposure in Gclm-null mice compared to their wild-type counterparts. (B) Loading scatter plot for differential ions between groups.
**Figure 2**: Metabolomics analysis showed depleted concentrations of glutathione and glutathione oxidized in *Gclm*-null mice with and without alcohol exposure.

**Glutathione Reduced**

**Glutathione Oxidized**

**Figure 3**: Targeted lipidomics analysis. Increased fold changes of triglyceride content in *Gclm*-null mice when compared to WT (A) as well as on alcohol exposure (B).