**Lysine methylation of PPARg in the regulation of lipid droplets formation and steatosis**

Hepatic accumulation of fat (steatosis) often occurs when the adipose tissue exceeds its storage capacity and lipids spill into the liver, leading to the development of fatty liver disease. Chronic liver disorders affect approximately 25% of the world's general population. The accumulation of fat is partly regulated by a set of transcription factors from the PPAR family. Specifically, PPARg is the main isoform that regulates lipid homeostasis in the liver. Histone methylation has been demonstrated as a central epigenetic mechanism to regulate steatosis, **highlighting the potential role of protein lysine methylation as a central post-translational modification governing fatty liver diseases*.*** Methylation of lysine residues is catalyzed by protein lysine (K) methyltransferases (PKMTs). The lysine acceptor residue serves as a scaffold for the recruitment of regulatory factors termed readers, which transduce downstream signaling pathways. Intriguingly, PKMT substrates are not confined to histones. In recent years the **methylation of non-histone proteins** has emerged as an essential modification that impacts diverse cellular signaling processes. **However, whether the methylation of non-histone proteins contributes to the initiation and progression of steatosis is currently unknown.**

 Our preliminary data suggest that the PKMT SETD6 is over-expressed in obese patients with BMI higher than 30. These patients display a significant accumulation of serum markers that have previously been identified as strong risk factors for impaired liver function. RNA-seq experiments in hepatocyte cell line (HepG2) have revealed that depletion of SETD6 correlates with the regulation of lipid metabolism processes and enrichment of the PPAR signaling pathway. At the molecular level, we have obtained preliminary data suggesting that SETD6 methylates PPARg at K170. By utilizing a live cell imaging approach, we found that SETD6 positively regulates lipid droplet formation, the primary storage organelles for neutral lipids in the liver. These data provide the ground for our **specific hypothesis that SETD6-mediated methylation of PPARγ positively regulates lipid droplet formation and steatosis.** In **Aims 1 and 2**, we will employ cellular, biochemical, and microscopic tools to specifically understand the molecular mechanisms of how PPARγ methylation by SETD6 regulates lipid droplet dynamics and hepatic steatosis. We will perform these experiments in different physiological and pathological settings using several hepatic cell lines, primary hepatocytes, and mice models for steatosis. In **Aim 3**, we will apply genomic approaches to determine how the methylation of PPARgalters transcriptional programs associated with lipid droplet formation and steatosis. In addition, we will characterize a potential positive feedback loop transcription mechanism between SETD6 and PPARg. This working hypothesis is based on preliminary data suggesting that the SETD6 promoter is enriched with several putative PPARg binding sites.

The current proposal brings together two fields of research – methylation signaling and hepatic lipid metabolism – and its successful completion will have an immediate impactand broad implications for both basic and translational research. In particular, it will challenge the possibility that SETD6-PPARg methylation-based signaling could act as an intervention target to limit lipid droplet formation and steatosis. Overall, we hope that our results will inform regarding new aspects of liver disease biology. This work will provide fundamental insights into the role of lysine methylation in regulating steatosis and identify possible targets for therapeutic intervention and translational applications.