Abstract: 10S

New insights to ER stress lipid and glucose metabolism: From NASH to insulin resistance and back

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NASH is a serious metabolic disease that can result in liver failure and a substantial increase in cardiovascular disease (CVD) and hepatocellular carcinoma (HCC) risk. The pathogenesis of NASH is poorly understood but it was explained by the multiple hit model, in which the first hit is hepatosteatosis with subsequent hits, including ER stress and endotoxemia, leading to a necroinflammatory response and steatohepatitis. Hepatosteatosis can be caused by elevated de novo lipogenesis (DNL), increased lipid import and defective lipid export. Most patients suffering from hepatosteatosis, or non-alcoholic fatty liver (NAFL), never progress to NASH giving rise to the notion that NAFL and NASH are distinct entities and not different stages in a linearly progressing disease, NAFLD. Recent data add strong support to this notion.

The major transcriptional activators of DNL, cholesterol biosynthesis and lipid import are SREBP1 and 2. In seminal work carried out by Horton, Brown, and Goldstein it was shown that hepatocyte-specific ablation of SREBP Cleavage Activating Protein (SCAP) abolishes SREBP1 and 2 expression and prevents hepatosteatosis in mice fed high fat diet (HFD) and genetically obese *ob/ob* mice. Neither of these mice develop NASH, thus representing a state akin to human NAFL. To study NASH in mice we developed a new model based on feeding MUP-uPA mice, which are highly susceptible to hepatocyte ER stress due to overexpression of uPA, with HFD or fructose enriched diets (HFrD and fructose drink supplemented HFD or HFHFD). Unlike HFD-fed BL6 mice or *ob/ob* mice, HFD-fed MUP-uPA mice show classical NASH signs, including Mallory Denk bodies, ballooning hepatocytes, hepatocyte death, inflammation, and fibrosis, after HFD, HFrD or HFHFD feeding. NASH development in MUP-uPA mice is accompanied by extensive ER stress and strong SREBP1 and 2 activation. We found that SREBP activation in these mice is SCAP independent due to marked induction of the SCAP inhibitor INSIG2. Instead, SREBP activation depends on cleavage of site 1 protease (S1P) by caspase-2, the catalytic component of the PIDDosome complex. Ablation of caspase-2 or the two other PIDDosome subunits, PIDD and RAIDD, which are needed for caspase-2 activation, blocks SREBP activation and NASH development. Curiously, instead of being protective, hepatocyte-specific SCAP ablation in MUP-uPA or BL6 mice strongly aggravates NASH development, enhancing liver damage and fibrosis, while decreasing steatosis, in response to HFD (MUP-uPA) and HFrD or HFHFD (BL6) feeding. Aggravated NASH is due to extensive ER stress and activation of IRE1, which promotes caspase-2 translation and SREBP1/2 degradation via the ERAD pathway. Treatment of the above mice with a IRE1 inhibitor blocks NASH development. Similar findings were made by Hayato Nakagawa's group who ablated SCAP in hepatocyte *Pten* knockout mice. They showed that liver damage is also propagated by ER stress driven by lipid imbalance. Dietary restoration of phospholipids or SREBP reactivation protected $Scap^{\Delta Hep}/Pten^{\Delta Hep}$ mice from NASH, providing strong evidence that hepatosteatosis may protect from NASH development.

A major driver of hepatosteatosis is insulin, whose main function is to reduce blood glucose. We recently made the novel discovery that FBP1 (fructose bisphosphate phosphatase), a rate limiting enzyme for gluconeogenesis (GNG), has another highly important regulatory function that does not depend on its enzymatic activity. FBP1 serves as a lynchpin that assembles a multiprotein complex that also contains PP2A-C and ALDOB, that binds AKT to prevent its overactivation by insulin. FBP1 deficiency in humans (mainly in infants) or mice can result in severe hypoglycemia, lactic acidosis, hepatomegaly, hepatosteatosis, liver damage and hyperlipidemia. However, regulated disruption of the FBP1: PP2A-C: ALDOB: AKT complex leads to complete reversal of obesity-induced insulin resistance.