Macrophage ATG16L1: potential candidate for NASH treatment

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Non-alcoholic steatohepatitis (NASH), also known as metabolic dysfunction-associated steatohepatitis (MASH), is becoming prevalent around the world. It has been estimated that around 5% of population are affected by NASH, and 1-2% of NASH patients will further progress to cirrhosis, which is a key risk factor for hepatocellular carcinoma (HCC), threatening life (1-3). Recently, FDA approved the first drug, Rezdiffra, a thyroid hormone receptor-beta (THRβ) agonist, for NASH treatment, bringing hopes to drug development in the field (4). However, as Rezdiffra is observed to be effective in only 25-30% of patients and the mechanism underlying THRβ activation reduced fibrosis is unknown, there is still unmet need for NASH pharmacotherapy (4).

NASH is characterized by steatosis, hepatocyte ballooning and lobular inflammation with different fibrosis stages (5, 6). Steatosis originates from lipid accumulation. The overload of lipid increases hepatic oxidative stress and induces mitochondria DNA (mtDNA) damage and cell death (7). The damaged hepatocyte will further activate liver resident macrophage, known as Kupffer cells, leading to the secretion of pro-inflammatory cytokines, including tumour necrosis factor (TNF), interleukin (IL)-1β and IL-6, which could recruit circulating monocytes to infiltrate the liver and differentiate into macrophages to amplify liver inflammation (8). Both injured hepatocytes and activated macrophages may induce the activation of hepatic stellate cells (HSCs) through either secreted proteins (Osteopontin, Indian hedgehog, etc.) or cytokines such as transforming growth factor-β (TGFβ) (9-11). Activated HSCs transdifferentiate into myofibroblasts
and synthesize fibrogenesis-related proteins, resulting in extracellular matrix deposition and fibrosis. Collectively, the development of NASH is determined by multiple hits involving different liver cell types.

Autophagy is an important biological process regulating cellular homeostasis by engulfing cytoplasmic components and delivering them to the lysosome for degradation, which provide amino acids, lipids and carbohydrates for cell survival (12, 13). There are three types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Macroautophagy starts with the induction of phagophore, which is elongated by autophagy-related genes (ATG) to engulf the cargo and form the autophagosome. The autophagosome is further transported and fused with the lysosome to degrade the sequestered material (12, 13). Unlike macroautophagy, microautophagy is mediated by the lysosome directly uptaking cellular material, while CMA is mediated by chaperone recognizing substrate proteins and translocating them to the lysosome (14). The role of autophagy in liver metabolism has been well studied in recent years with the help of ATG5 or ATG7 inhibition tools or conditional knockout mice (15). It has been found in hepatocytes, blocking autophagy by ATG7 deletion or ATG5 knocking down leads to lipid accumulation, and in macrophages, ATG5 deletion increases monocyte infiltration and liver inflammation, causing fibrosis (16, 17). Contrary to the beneficial effect of autophagy in hepatocytes and macrophage, deletion of ATG7 in HSCs results in attenuated fibrosis (18). While lipid accumulation, liver inflammation and fibrosis are the main features of NASH, and the above studies have indicated there might be a tight correlation between autophagy dysfunction and NASH pathogenesis, there are few studies verifying if disrupting ATG proteins will affect NASH.

To answer this question, in the current issue of Clinical and Molecular Hepatology, Wang et. al first examined ATG16 like protein 1 (ATG16L1) expression in the liver of NASH patients and observed a significant decrease of ATG16L1 specifically in macrophage (19). ATG16L1 is part of the ATG12-ATG5-ATG16L conjugation complex and participates in the elongation of the phagophore to form the phagosome in macroautophagy. The reduced expression of ATG16L1 may lead to an inhibition of autophagy. The authors next generated macrophage specific Atg16l1 knockout or overexpression mice to determine if altering ATG16L1 expression would affect the NASH phenotype induced by a high-fat and high-cholesterol diet (HFHCD) or methionine-choline deficient (MCD) diet-feeding. Intriguingly, macrophage ATG16L1
knockout exacerbates NASH with increased lipid content, inflammatory factors expression and liver fibrosis, while overexpression of ATG16L1 in macrophage ameliorates NASH with fibrosis.

This is due to a complex mechanism determined by the authors in the following study. When mtDNA released from injured hepatocytes in NASH liver, it is up-taken by macrophages, activates the stimulator of interferon genes (STING) signaling pathway and increases the expression of proinflammatory genes through either TANK-binding kinase-1 (TBK1)- interferon regulatory factor-3 (IRF3) or NF-κB signaling pathway. In ATG16L1 knockout macrophages, autophagy and the degradation of lipid droplets (LDs)-lipophagy- are inhibited, resulting in less lipid peroxidation product-4-hydroxynonenal (4-HNE). Reduced 4-HNE increases the carbonylation and blocks the palmitoylation of STING, which promotes STING translocation and activation, leading to increased inflammation in the liver of macrophage Atg16l1 knockout mice under NASH condition. The increased proinflammatory genes expression in macrophage Atg16l1 knockout mice further augmented hepatocyte lipid accumulation. Besides, the activation of STING signaling pathway also increases TGFβ expression through the transcription factor c-JUN/FOS in the ATG16L1 deleted macrophages, which contributes to the activation of HSCs and fibrosis.

Collectively, this study proved a novel function of macrophage ATG16L1 in NASH, and peretionin-an ATG16L1 enhancer- shows promising results in reversing almost all of the NASH phenotype induced by HFHCD or MCD diet feeding in mice, suggesting a potential candidate for NASH treatment. There are some interesting findings in this study, however, still left to be discussed. For example, the body weight of macrophage Atg16l1 knockout mice is significantly increased with less energy expenditure after HFHCD feeding compared to the control group. As Lyz2-Cre is expressed in all macrophages, the contribution of macrophage ATG16L1 from other tissues such as white adipose tissue (WAT) in NASH may need more investigation.
References: