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Activation of AMPK for a Break in Hepatic Lipid Accumulation and Circulating Cholesterol

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Dysregulation of lipid metabolism leading to excessive lipids within the liver constitutes a major risk factor for life-threating diseases, such as non-alcoholic fatty liver disease (NAFLD) as well as obesity and diabetes, and atherosclerotic cardiovascular disease. NAFLD represents the most common chronic liver disease and encompasses a wide range of liver abnormalities, from benign simple steatosis (ectopic accumulation of lipids), potentially progressing to an intermediate form, nonalcoholic steatohepatitis with inflammation (NASH), and to advanced stages of liver disease, including fibrosis, cirrhosis, and, in some cases, hepatocellular carcinoma. Currently, therapeutic approaches are limited and no pharmacological treatments approved by regulatory agencies are available. Accumulation of hepatic triglycerides (TGs) in NAFLD was estimated to derive for ~60% from fatty acids released from adipose tissue and for ~25% from de novo lipogenesis (DNL) [1]. A valuable treatment option is targeting of the evolutionarily conserved energy sensor AMP-activated protein kinase (AMPK) originally identified as the kinase that phosphorylates and inhibits both acetyl coenzyme A carboxylase (ACC) and 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMGCR), which are the rate-limiting enzymes for fatty acid and cholesterol biosynthesis, respectively [2]. Activation of AMPK in the liver by a number of pharmaceutical/nutraceutical compounds has been reported to inhibit fatty acid synthesis and promote fatty acid oxidation (FAO) via phosphorylation and inactivation of ACC [3–5]. Because of these effects, AMPK hold promise as an attractive therapeutic target to rescue the liver from excessive lipid accumulation and limit possible risk of NAFLD progression.

Mammalian AMPK exists as a heterotrimeric complexes comprising a catalytic α subunit and regulatory β and γ subunits that occurs as multiple isoforms (α1, α2, β1, β2, γ1, γ2, γ3) with unique tissue-specific expression profiles. AMPK plays a key role in controlling cellular energy balance and metabolism by turning off ATP-consuming anabolic pathways (e.g., lipid synthesis) and switching on catabolic pathways (e.g., fatty acid oxidation) in response to metabolic stresses [2]. Since AMPK is sensitive to changes in cellular nucleotide levels (AMP:ATP and ADP:ATP ratios), AMP mimetics and compounds modulating cellular energy charge (e.g., by inhibition of ATP synthesis via inhibition of mitochondrial function) were attractive AMPK-activating treatments. However, most of these compounds activate AMPK indirectly and have AMPK-independent off-target pharmacological effects, thus limiting enthusiasm for clinical application and development [5]. Recent crystallographic studies helped to identify a novel allosteric site, termed as allosteric drug and metabolite (ADaM) site, corresponding to a pocket formed between the β subunit and the kinase domain of the α subunit [2]. Identification by Abbott Laboratories of the first direct AMPK activator A-769662 binding the ADaM site was an important pharmacological breakthrough that encouraged large screening programs to identify novel direct AMPK activators [6,7].

In this issue of EBioMedicine, Esquejo and colleagues [8] describe the therapeutic effect of a novel direct AMPK β1 biased activator (PF-06409577) on NAFLD progression in mouse and rat models. In rodent liver, α1β1γ1 and α2β1γ1 heterotrimers are predominantly expressed and it was not surprising that PF-06409577 suppresses DNL in wild type but not AMPKα1/α2-deficient primary mouse hepatocytes, as previously shown with A-769662 specific for β1-containing complexes [3]. However, it was important to demonstrate the ability of this AMPK β1 biased activator to activate AMPK in human hepatocytes because α1β2γ1 has been identified as the major heterotrimer [3]. Despite low levels of AMPK β1 subunit in human hepatocytes, the authors show a dose-dependent inhibition of DNL with an EC50 of 128 nM compared to 49 nM in rat hepatocytes [8]. Similar results were found with previous AMPK β1 biased activators [3]. In addition, consistent with AMPK-dependent phosphorylation and inactivation of ACC, PF-06409577-induced suppression of fatty acid synthesis was severely blunted in hepatocytes isolated from mice with alanine knock-in mutations (ACC-DKI) that render ACC activity insensitive to AMPK [8]. Chronic PF-06409577 treatment in obese rodents resulted in reduction of liver TG content in wild type but not liver AMPKα1/α2 KO mice, excluding significant contribution of AMPK activation in non-hepatic tissues. This lipid-lowering effect was accompanied by an increase in circulating β-hydroxybutyrate levels in wild type but not liver AMPKα1/α2 KO mice, suggesting a contribution of FAO in addition to the inhibition of DNL. In support of the importance of AMPK activation and ACC phosphorylation in the stimulation of FAO, recent studies reported that a

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large panel of AMPK activators failed to induce FAO in AMPKα1/α2 KO and ACC-DKI hepatocytes [3,4].

Interestingly, the authors find that regulation of hepatic lipogenesis by AMPK activation mainly resides in the phosphorylation and inactivation of ACC but not in the control of lipogenic gene expression, in accordance with previous study [3]. Overall, these findings suggest therapeutic potential of direct targeting ACC to lower hepatic fat. This was recently tested in a small human trial where administration of a pharmacological inhibitor of ACC to subjects with hepatic steatosis led to a significant reduction in liver TGs, but this was also associated with hypertriglyceridemia, precluding further clinical development [9]. An alternative approach to lower DNL in humans could be through the activation of AMPK which is not accompanied with a rise in plasma TGs in preclinical animal models [7,8].

There has been increasing interest in finding new therapeutic strategies that could prevent or even reverse established hepatic fibrosis and progression to NASH. Encouraging findings from this study is the alteration of the hepatic fibrosis/stellate cell activation pathways in response to chronic AMPK activation. Although, these data are interesting, the impact of chronic AMPK activation should be confirmed in relevant models of fibrosis. In addition, the mode of action of AMPK activation is elusive and remains to be elucidated.

The study from Esquejo et al. also addresses the consequence of AMPK activation on the regulation of cholesterol metabolism. The authors show that PF-06409577 treatment lowers plasma mevalonic acid levels, a direct product of HMGCR and resulted in the reduction of plasma cholesterol and increase of plasma HDL cholesterol in ZSF1 rat, a model mimicking human hypercholesterolemia. Although clinical relevance was provided by a reduction of circulating plasma cholesterol and LDL in cynomolgus monkeys treated for 6 weeks with PF-06409577, these data raise important questions about the role for AMPK and the potential target proteins and tissues involved in these benefical effects. Analysis of mice with alanine knock-in mutation of the AMPK phosphorylation site in HMGCR could be an interesting area of future studies. Lastly, long-term studies addressing the efficacy of AMPK β1 biased activators for the prevention of atherosclerotic cardiovascular disease, alone or when used in combination strategies with current LDL-cholesterol lowering drugs (e.g., statins, PCKS9 inhibitors), are warranted.

Collectively, these results provide compelling evidence for the therapeutic potential of AMPK activation in the treatment of hepatic lipid disorders. The authors not only confirmed that AMPK activation improves hepatic lipid content in obese rodents but also showed a reduction of cholesterol plasma concentration in a hypercholesterolemic rat model and in non-human primates. Therefore, these findings open interesting therapeutic perspectives for the use of novel generation of small molecule AMPK activators for the treatment of dyslipidemia. In particular, the insights afforded by this study are truly exciting and will pave the way to new therapeutic strategies to mitigate or prevent the development of NAFLD, hyperlipidemia and the risk of associated complications (e.g. NASH, atherosclerotic cardiovascular disease). Thus, direct AMPK activators warrant further testing as a treatment of NAFLD in humans. However, before consideration, benefits must be balanced against potential cardiac hypertrophy, as recently reported after long-term use of a pan-β AMPK activator in rats and rhesus macaques [10]. Although PF-06409577 entered a phase I clinical trial for the treatment of diabetic nephropathy, this study was not completed [6] and there is currently no available data on the possibility of safe therapeutics with biased or isoform-selective AMPK activators.

Disclosures

The authors declare no conflicts of interests.

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