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ASKing no more: the emerging role of DUSP12 in the regulation of hepatic lipid metabolism

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Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding conflicts of interest respect to this manuscript.

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Authors' contributions

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Editorial

Accumulation of fat in liver cells not due to alcohol abuse is the hallmark of non-alcoholic fatty liver disease (NAFLD), a common condition that may progress to non-alcoholic steatohepatitis (NASH) characterized by liver inflammation.[1] Over a long period of time, NASH may lead to fibrosis with consequent cirrhosis, which in turn predisposes patients to hepatocellular carcinoma.[1] One of the reasons that fat accumulated in the liver is harmful could be its occurrence with lipotoxicity, which causes oxidative and endoplasmic reticulum stress as well as mitochondrial dysfunction with the generation of reactive oxygen species (ROS), all leading to hepatocytes death and inflammation.[2] Because of the close association with obesity, NAFLD is increasingly becoming an epidemic. Indeed, NAFLD affects up to a third of the population in many industrialised countries and is usually diagnosed late when change in lifestyle and diet is not effective anymore.[1] Therefore, the only remedy may be pharmacological intervention or liver-directed pharmacotherapy. Among the signalling pathways involved in the pathogenesis of NAFLD are members of mitogen-activating protein kinase (MAPK) family, which includes three members: extracellular-regulated kinase (ERK1/2), Jun N-terminal kinase (JNK) and p38-MAPK.[3] JNK and p38 are stress-responsive pathways that are regulated by the upstream MAP3K apoptosis-signal-regulating kinase 1 (ASK1), which has been shown to be activated in murine models of hepatic steatosis and patients with NASH. This has recently led to the development of ASK1 inhibitors for the treatment of NASH.[4,5] While it is reasonable targeting the ASK1 pathway, results from Phase 3 studies of selonsertib (an investigational ATP-competitive ASK1 inhibitor) in patients with NASH and bridging fibrosis (STELLAR-3) and compensated cirrhosis (STELLAR-4) have shown neither improvement in fibrosis nor NASH amelioration. A further in-depth analysis of the molecular mechanisms that underlie ASK1 expression and activities in chronic liver disease may, therefore, led to a better therapeutic approach. In this issue of HEPATOLOGY, Huang and colleagues[6] report a very interesting study addressing the regulatory mechanisms underlying ASK1 activation. Using gain- and loss-of-function studies *in-vitro* and *in-vivo*, they show that DUSP12, a member of the dual specific phosphatase (DUSP) family, regulates hepatic lipotoxicity through inhibition of the ASK1 signal cascade (**Figure 1**).[6]

Phosphatases can catalyse the removal of phosphate groups from specific amino acid residues in target kinases (substrates) that can result in the kinases inactivation.[7] DUSPs play vital roles in inflammation and immune cells activation, diabetes and cell-growth signalling.[7] However, their mechanisms of action are still poorly understood. Huang et al.[6] show that in livers of mice fed a high-fat diet (HFD) the expression of DUSP12 is significantly lower than that in liver samples of normal lean animals. Compared to untreated immortalised hepatocytes DUSP12 expression levels are also lower in hepatocytes cultured in presence of palmitic acid and oleic acid (PAOA),

the most abundant fatty acids in western diets. Intriguingly, it has been recently shown that the expression of two related DUSP proteins, DUSP9 and DUSP26, are downregulated in liver of mice with hepatic steatosis,[8,9] underscoring the important role of the DUSP proteins in the pathogenesis of liver diseases.

To assess the functional role of DUSP12 in the pathogenesis of NAFLD, Huang et al.[6] have generated liver-specific DUSP12 knockout (*DUSP12*-CKO) mice and fed them with HFD to induce hepatic steatosis and obesity-related metabolic conditions. While these mice displayed no apparent increase in body weight compared to control diet-fed mice, liver weights were higher in HFD-fed *DUSP12*-CKO mice. The increase in liver weight was also associated with a remarkable increase in lipid accumulation, levels of plasma triglycerides, total cholesterol and non-esterified fatty acids (NEFAs) in HFD-fed *DUSP12*-CKO livers, suggesting that DUSP12 protects mice from developing hepatic steatosis. Consistent with this, *DUSP12*-CKO mice fed with a high-fat/high-cholesterol (HFHC) diet displayed an increase in hepatic lipid accumulation and enhanced expression of mRNA of enzymes involved in fatty acid synthesis compared to normal diet-fed mice. Yet, hepatic depletion of DUSP12 aggravates liver fibrosis and inflammation in response to HFHC diet. By using a liver-specific DUSP12 transgenic mouse model, the authors demonstrate that the overexpression of DUSP12 in hepatocytes leads to hepatic lipogenesis in response to HFD and results in decreased expression levels of inflammatory cytokines, confirming the essential role of DUSP12 in the regulation of hepatic inflammation and lipid metabolism.[6]

Mice that are fed a high-fat diet develop a variety of pathological conditions including obesity, diabetes, steatohepatitis and cardiovascular disorders.[1] Obesity is associated with a state of chronic, low-grade inflammation that increases the systemic levels of pro-inflammatory cytokines whose effects on targeted tissues contribute to generate insulin resistance, namely the inability of insulin to stimulate the transport of glucose into the cells.[1] Compared to control mice, lack of DUSP12 in livers of HFD-fed mice enhanced insulin resistance, accompanied by a reduction in mRNA levels of gluconeogenesis-related genes and an increase in the expression of pro-inflammatory cytokines and fasting blood glucose levels. This study is, therefore, of utmost importance as it emphasises the functional role of DUSP12 in the protection of livers from inflammation-mediated insulin resistance. Similar phenotypes were observed in either liver-specific DUSP9 or DUSP26 knockout mice fed with HFD. This begs the questions how the activities of different DUSPs are regulated, and how distinct DUSPs regulate the functions of their substrates. Although the search for an answer in the mechanisms of activation and regulation of DUSP12 remain to be elucidated, Huang et al.'s study reveals an unexpected mechanism by which DUSP12 inhibits the progression of NAFLD/NASH.[6]

Importantly, DUSPs are master regulators of the MAPK signalling cascade.[3,7] MAPKs are phosphorylated and activated via a cascade module in which MAP3Ks activates MAP2Ks that in turn activate MAPKs (**Figure 1**). While the ERK-MAPK signalling diverges from JNK and p38-MAPK at the MAP3K level, both JNK and p38-MAPK signalling cascade are activated by the MAP3K ASK1.[3] It was, therefore, logical for the authors to start analysing the effect of DUSP12 on MAPKs. Surprisingly, compared with control mice, liver samples of HFD-fed *DUSP12*-CKO mice displayed an enhanced phosphorylation of both JNK and p38-MAPK. Gain-of-function analyses using mice overexpressing DUSP12 in hepatocytes proved the contrary. Notably, DUSP12 depletion did not result in a significant change of ERK phosphorylation. The intriguing finding is that phosphorylation and activation of ASK1 (but not TBK1 – another related MAP3K) was found impaired in liver samples of HFD-fed *DUSP12*-CKO mice. This study also demonstrates that DUSP12 directly interacts with ASK1 (**Figure 1**). In contrast, an inactive form of DUSP12 (containing a point mutation in the catalytic domain) is unable to interact with ASK1, suggesting that DUSP12 activity is required for the DUSP12/ASK1 interaction. In addition, blocking the ASK1-JNK and ASK1-p38 cascade in immortalised hepatocytes reversed the lipid accumulation and the upregulation of genes involved in lipid metabolism and inflammation (**Figure 1**).

Together these data indicate that the DUSP12-ASK1 regulatory axis is an important determinant for the lipogenesis in hepatocytes and provide a molecular basis for the progression of NAFLD. Because the broad tissue expression profiles of MAPKs and their redundancy may limit the success of MAP3K inhibitors in the clinic,[7] further studies are needed to assess the potential benefits of DUSP12 activation in treating NASH. We would like to encourage investigators to extend the Huang *et al*.'s study by increasing, for example, DUSP12 activity which provides a 'natural' inactivation of hepatic ASK1. The different expression profiles and distinct preferences of DUSP proteins for their MAPK substrates[7] suggest that targeting DUSP12 may be an alternative strategy for manipulating ASK1 activity to treat liver steatosis and lipogenesis-associated diseases.

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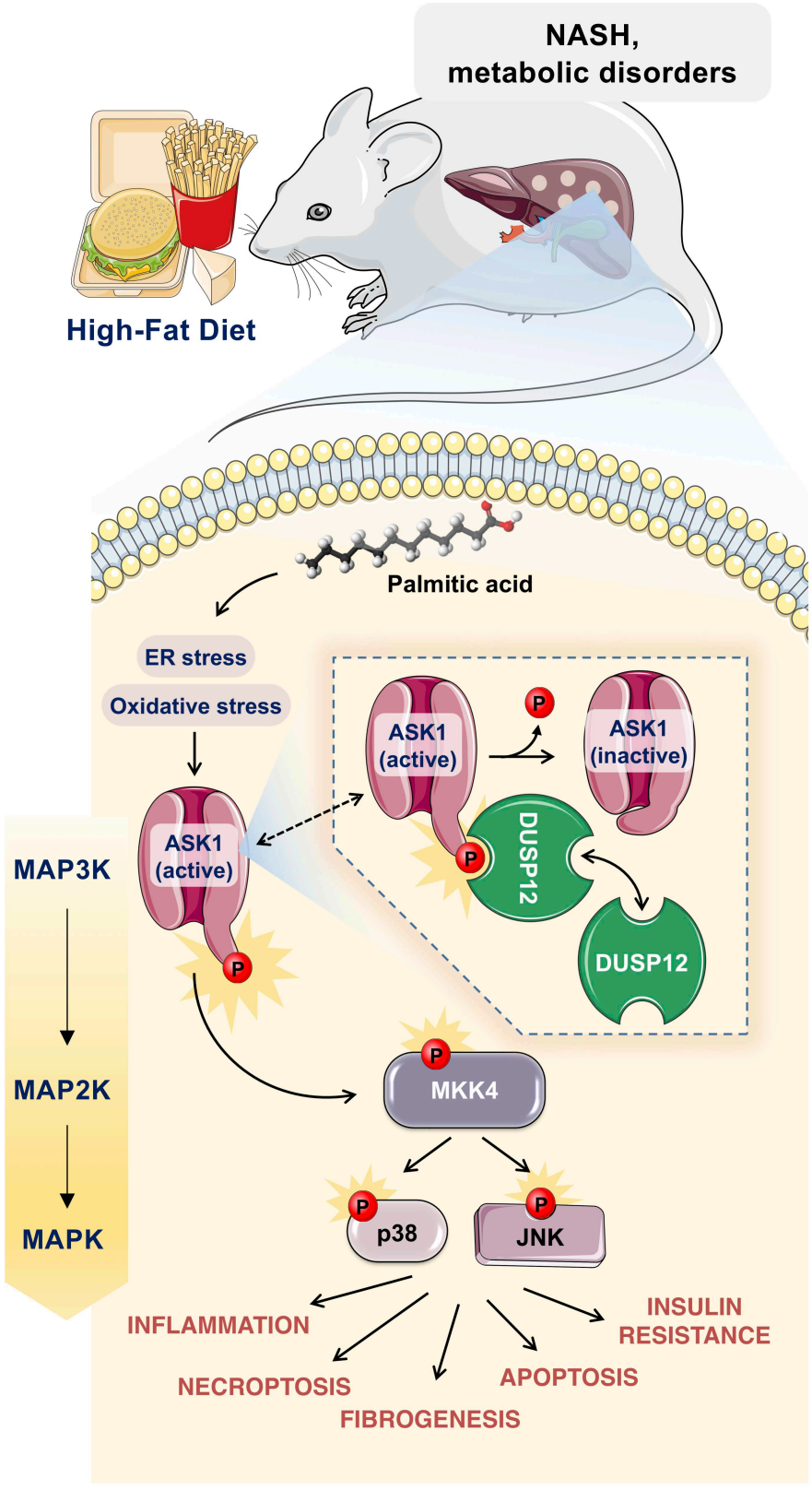
Figure Legends

Figure 1. Simplified representation of DUSP12 regulation of hepatic lipotoxicity through inhibition of ASK1 signal cascade. Schematic illustration depicting the activation of ASK1 in the liver in response to a high-fat diet during the development of NASH. The incorporation of saturated fat (i.e. palmitic acid) in hepatic cells promotes endoplasmic reticulum (ER) stress and oxidative stress that contributes to the phosphorylation and activation of the MAP3K ASK1. Like all other MAPK signalling cascades, activation of ASK1 lead to phosphorylation (P) and activation of the MAP2K components including MKK4, which in turn phosphorylates and stimulates the activity of distinct MAPKs such as JNK and p38 protein kinases. Upon activation, each MAPK itself can phosphorylate specific substrates delivering different cellular activities including apoptosis and necroptosis. In the study presented by Huang and colleagues, the authors propose a regulatory model whereby the constitutive expression and activation of the protein phosphatase DUSP12 maintains ASK1 in an 'inactive' form (dotted line). Mechanistically, the authors show that DUSP12 binds and dephosphorylates ASK1 which results in the suppression of ASK1-JNK and ASK1-p38 signalling cascade. This is sufficient to protect livers from lipotoxicity induced by high-fat diets. Genetic depletion of DUSP12 in mouse livers, on the other hand, unleashes 'active ASK1' to phosphorylate MKK4 enhancing its kinase activity necessary for the phosphorylation of both p38 and JNK, thus promoting apoptosis, fibrogenesis and NASH.


NASH,
metabolic disorders
High-Fat Diet
Palmitic acid
ER stress
Oxidative stress
ASK1
(active)
P
ASK1
(inactive)
DUSP12
DUSP12
ASK1
(active)
P
MAP3K
MAP2K
MAPK
P
MKK4
P
p38
P
JNK
INFLAMMATION
INSULIN
RESISTANCE
NECROPTOSIS
APOPTOSIS
FIBROGENESIS