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MAPK1/3 regulate hepatic lipid metabolism via ATG7-dependent autophagy

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ABSTRACT

Although many biological functions of MAPK1/ERK2-MAPK3/ERK1 (mitogen-activated protein kinase 1/3) have been reported, a direct effect of MAPK1/3 on hepatic lipid metabolism remains largely unknown. We recently showed that activation of MAPK1/3 ameliorates liver steatosis in LEPR (leptin receptor)-deficient (*db/db*) mice, a classic animal model for liver steatosis. Consistent with these results, knockdown of MAPK1/3 promotes liver steatosis in C57/B6J wild-type (WT) mice. Autophagic flux and ATG7 (autophagy related 7) levels are increased by MAPK1/3 activation or decreased by MAPK1/3 knockdown in livers and primary hepatocytes. Blockade of autophagic flux by chloroquine (CQ) or ATG7 knockdown reverses the ameliorated liver steatosis in MAPK1/3-activated *db/db* mice. Together, these findings identify a beneficial role for MAPK1/3 in liver steatosis that is mediated by ATG7-dependent autophagy, which provides novel insights into the mechanisms underlying liver steatosis and create a rationale for targeting MAPK1/3 in the treatment of liver steatosis.

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Non-alcoholic fatty liver disease encompasses a series of pathological changes in liver. The first stage of non-alcoholic fatty liver disease is liver steatosis, which is characterized by the excess deposition of triglyceride (TG) and/or cholesterol. Liver steatosis develops as a result of increased de novo lipogenesis, augmented fatty acid uptake, decreased fatty acid oxidation and/or impaired TG export.

Autophagy, a cellular process through which cells engulf and degrade damaged cytoplasmic components, has recently been reported to play an important role in the regulation of hepatic lipid metabolism. Impaired autophagy decreases hepatic fatty acid β-oxidation and TG export, which results in liver steatosis. MAPK1/3 is reported to play a role in autophagy and several lines of evidence imply a link between MAPK1/3 and lipid metabolism. Whether MAPK1/3 regulates hepatic lipid metabolism via autophagy, however, remains largely unknown. Our current work demonstrates that MAPK1/3 plays a beneficial role in hepatic lipid metabolism by stimulating ATG7-dependent autophagy.

To determine whether MAPK1/3 has a direct effect on hepatic lipid metabolism, we examined MAPK1/3 activity in the livers of C57/B6J WT and *db/db* mice and found that MAPK1/3 phosphorylation, an indirect measurement of MAPK1/3 activity, is compromised in the livers of *db/db* mice. Injecting *db/db* mice with adenoviruses expressing constitutively active MAP2K1/MEK1 (Ad-CA MAP2K1), the specific upstream activator of MAPK1/3, largely ameliorates liver steatosis, as shown by H&E and Oil Red O staining, and liver TG measurements. By contrast, injection of control adenoviruses expressing green-fluorescent protein (Ad-GFP) have no effect. The improvement of liver steatosis in *db/db* mice injected with Ad-CA MAP2K1 results from an enhancement of fatty acid β-oxidation and TG export, as shown by the increased expression of genes related to these processes and increased serum β-hydroxybutyrate levels. Opposite effects were observed when MAPK1/3 was knocked down by adenoviruses expressing small-hairpin RNA against MAPK1/3 (Ad-sh*Mapk1/3*) in C57/B6J WT mice.

Because autophagy regulates hepatic lipid metabolism by accelerating fatty acid β-oxidation and TG export and MAPK1/3 plays a role in autophagy, it is likely that MAPK1/3 regulates hepatic lipid metabolism via autophagy. Consistent with this possibility, we found that autophagic flux is enhanced by MAPK1/3 activation and inhibited by MAPK1/3 knockdown both in livers and primary hepatocytes, as demonstrated by corresponding changes in protein levels of the autophagy markers SQSTM1 and LC3-II, and by an autophagic flux analysis. The possible involvement of autophagy in MAPK1/3 regulation of liver steatosis in *db/db* mice was further confirmed by the observation that the ameliorated liver steatosis in *db/db* mice by Ad-CA MAP2K1 injection is reversed by treatment with the autophagy inhibitor chloroquine, as shown by H&E and Oil Red O staining, and liver TG measurements.

It is well known that autophagy is a dynamic process regulated by many autophagy-related regulators. For this reason, we

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examined mRNA levels of autophagy-related (*Atg*) gene regulators, including *Atg4a, Atg5, Becn1* and *Atg7* in livers and primary hepatocytes following MAPK1/3 activation or knockdown. This survey showed that only ATG7 expression is influenced by the state of MAPK1/3 in a manner consistent with changes in hepatic TG accumulation in both livers and hepatocytes.

To investigate whether MAPK1/3 regulates hepatic lipid metabolism via ATG7-dependent autophagy, we injected Ad-CA MAP2K1 *db/db* mice with adenoviruses expressing small-hairpin RNA against ATG7 (Ad-sh*Atg7*) or control scrambled adenoviruses. These experiments showed that the ameliorated liver steatosis and enhanced autophagic flux in Ad-CA MAP2K1 *db/db* mice is reversed by Ad-sh*Atg7* injection as measured by H&E and Oil Red O staining, TG measurements and changes in protein levels of autophagy markers. Consistently, the promoted liver steatosis and inhibited autophagic flux in Ad-sh*Mapk1/3* WT mice is reversed by the injection of adenoviruses expressing ATG7 (Ad-ATG7).

Interestingly, phosphorylation of MAPK/p38, which acts as an inhibitor of autophagy, is increased by MAPK1/3 knockdown and decreased by MAPK1/3 activation in both livers and primary hepatocytes. Treatment of primary hepatocytes with the MAPK/p38-specific inhibitor SB203580 or transfection with *Mapk14/p38α*-specific siRNA reverses the effects of MAPK1/3 knockdown on the inhibition of ATG7 expression and autophagic flux. The role of MAPK/p38 in MAPK1/3-regulated autophagy and hepatic lipid metablism in vivo, however, needs to be explored further in the future.

Taken together, our findings identify a novel function of MAPK1/3 in regulating hepatic lipid metabolism via ATG7-dependent autophagy. These results provide new insights into the physiological role of MAPK1/3 in liver and a theoretical basis for activating MAPK1/3 as a potential treatment target for liver steatosis.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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