Published in final edited form as:

Hepatology. 2018 January ; 67(1): 438–441. doi:10.1002/hep.29495.

Tmbim 1, a novel anti-inflammatory approach for NASH treatment

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Keywords

Tmbim1; TLR4; cell death; lysosomal degradation; non-alcoholic fatty liver disease

The worldwide prevalence of nonalcoholic fatty liver disease (NAFLD) has reached 20–40%. Approximately 10–20% of NAFLD cases progress to nonalcoholic steatohepatitis (NASH), which is a hepatic pathology characterized by aberrant lipid accumulation, hepatocellular damage, inflammation and fibrotic scarring in the liver..(1) Moreover, development of NAFLD and NASH is closely associated with increased circulating endotoxin levels in both human and animals. The recognition of LPS, as well as other danger associated molecular patterns, by Toll-like receptor 4 (TLR4) expressed in both hepatocytes and non-parenchymal cells of the liver leads to liver injury and fibrosis through the induction of oxidative stress as well as the production of proinflammatory cytokines..(2) Intracellular protein degradation via the lysosome or the ubiquitin-proteasome system has been recognized as an attractive platform for drug targeting, and proteolysis-based agents targeting mainly the latter have been developed as clinical treatments for a variety of cancers.(3) The transmembrane BAX inhibitor motif-containing 1 (Tmbim1; also known as Recs1) is a membrane protein that localizes to late endosomes and lysosomes. Known functions of Tmbim1 include inhibition of Fas-mediated apoptosis, maintenance of cellular Ca²⁺ homeostasis and suppression of matrix metalloproteinase production (4)

Zhao et al. (5) recently reported on a previously unrecognized role of Tmbim1 as a regulator of the multivesicular body (MVB)-lysosomal pathway of protein degradation and as a key suppressor of steatohepatitis by facilitating the lysosomal degradation of Tlr4. Their studies were able to demonstrate that overexpression of Tmbim1 in the liver, using an adeno-associated virus 8 (AAV8) vector, effectively relieved the progression of NAFLD in mice and suppressed steatohepatitis and metabolic syndrome (MetS) in monkeys. Their study sets base for a promising new therapy for NASH via targeting MVB regulators to orchestrate the lysosome-mediated protein degradation of key mediators of the disease process.

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Conflict of Interest: The authors state no conflict of interest.

In a set of elegant experiments involving microarray analysis comparing gene expression in livers of mice that were given either a high-fat diet (HFD) or a normal chow diet revealed that reduced *Tmbim1* expression levels are associated with fatty liver disease, higher fasting blood glucose and fasting serum insulin concentrations. Developing hepatocyte-specific *Tmbim1*-knockout mice enabled the group to discover, that hepatocyte *Tmbim1* deficiency significantly increased the ratio of liver weight to body weight, hepatic lipid droplets, including triglycerides and non-esterified fatty acids, as well as the activity of inflammatory signaling mediated by the transcription factor NF- κ B. Next molecular mechanisms underlying the functions of *Tmbim1* were explored, which highlighted *Tlr4* as a key factor for *Tmbim1* function. *Tlr4* protein expression was markedly upregulated by *Tmbim1* deletion and downregulated by *Tmbim1* overexpression both in vivo and in vitro. When hepatocyte-specific *Tlr4*-knockout (*Tlr4*-HepKO) mice were crossed with *Tmbim1*-knockout mice the *Tmbim1*-deficiency-induced exacerbation of insulin resistance, hepatic lipid accumulation, inflammatory response and liver injury was reversed indicating that *Tmbim1* attenuation of steatohepatitis was dependent on induction of *Tlr4* degradation.

To evaluate the effects of TMBIM1 in a more clinically translatable model, authors used the nonhuman primate *Macaca fascicularis*. Primates were fed a HFD for 32 weeks to promote the development of NASH and MetS. AAV8 encoding human TMBIM1 was successfully delivered to the livers of monkeys at the beginning of the HFD treatment and substantially reduced the HFD-induced increases in fasting blood glucose and fasting insulin levels. Moreover, HFD-triggered increase in serum alanine aminotransferase (ALT) levels was largely blocked by the overexpression of TMBIM1, without a significant influence on the increased aspartate aminotransferase (AST) levels. In the liver, lipid accumulation, inflammation and fibrosis were all present at significantly lower levels in AAV8-TMBIM1-injected monkeys than in AAV8-Control-injected monkeys. Body weight, body mass index, waist circumference, caloric intake and blood pressure were not significantly affected by TMBIM1 overexpression. The findings by Zhao et al. collectively provide strong support for the promising application of TMBIM1 as a therapeutic target in NASH and metabolic disorders (Fig. 1).

As previously mentioned, TLR4 is found both in hepatocytes and non-parenchymal cells of the liver including macrophages (Kupffer cells) and stellate cells and its expression increases in the liver of mouse models and patients with NASH. While experimental studies have shown genetic or pharmacological inhibition of TLR4 can prevent or protect from key components of NASH mainly liver inflammation and fibrosis, due to the central role of TLR4 in innate immunity, blocking it raises infection risk concerns.(6)

TMBIM1 belongs to a highly conserved protein family of key regulators of cell death with anti-apoptotic activity.(4) TMBIM1 forms a complex with Fas/CD95/Apo1 and specifically reduces Fas ligand (FasL)-induced apoptosis a pathway that has been implicated in the pathogenesis of NASH.(7) This additional mechanism was not explored in the present study and will require further examination. In addition, all studies were conducted using genetic approaches to modulate TMBIM1 specifically in hepatocytes and thus it will be important in the future to see the effects of activating TMBIM1 in tissues beyond hepatocytes.

The same group of investigators have recently reported on another emerging target for NASH based on the Casp8 and Fadd-like apoptosis regulator (Cflar) and its small segment Cflar(S1), which function by blocking the kinase Map3k5 (also known as Ask1), a target that has already been identified for the treatment of NASH.(8) ASK1 activates the JNK and p38 MAPK pathways triggering apoptosis, the secretion of inflammatory cytokines, and induction of fibrogenic genes key cellular and molecular mechanism involved in NASH progression. The targeting of TMBIM1–TLR4 and CFLAR–ASK1 represent distinct molecular mechanisms in NASH treatment and thus would produce different therapeutic approaches and drug candidates. Certain downstream effectors of these targets (for example, JNK and NF- κ B signaling) and the phenotypes (for example, hepatic steatosis and insulin resistance) are shared by TLR4 and ASK1. Therefore, future studies are warranted to access the relative efficacy and safety of these approaches and whether the simultaneous targeting of both TMBIM1–TLR4 and CFLAR–ASK1 might be more advantageous than targeting either one alone.

Acknowledgments

Funding: This work was funded by NIH grants R01 DK082451, U01 AA022489 to AEF. AW is supported by the START-Program of the Faculty of Medicine, RWTH Aachen, and DFG grant WR173/3-1 and SFB/TRR57.

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Tmbim1 based therapy

TLR4-mediated progression

Fig. 1.

Tibim1 reduces TLR4-dependent liver damage. A wide range of stimuli can activate TLR4 receptor on hepatocytes activating inflammatory signaling and liver disease progression. Endosomal TLR4 recycling perpetuates liver inflammation and liver disease progression. Tmbim1 functions as a regulator of multivesicular body lysosomal pathway and as a key suppressor of steatohepatitis by facilitating the lysosomal degradation of Tlr4.