Corrected: Author Correction

METABOLIC LIVER DISEASE IN 2018

Relieving ER stress to target NASH-driven hepatocellular carcinoma

Saskia Reibe and Mark A. Febbraio

A key component in the development from fatty liver to hepatocellular carcinoma (HCC) is the appearance of nonalcoholic steatohepatitis (NASH). The precise cellular processes that trigger the advancement of NASH towards HCC are not well understood. In 2018, three key papers were published that help us better understand these processes.

One organ that is highly challenged by the current obesity pandemic is the liver. The hepatic endoplasmic reticulum (ER) is pivotal as it processes many aspects of lipid metabolism[1] and is clearly linked to the development of hepatocellular carcinoma (HCC)[2]. The exact steps involved are poorly understood, but one hallmark of the path from simple steatosis to HCC is the development of nonalcoholic steatohepatitis (NASH), a severe form of nonalcoholic fatty liver disease (NAFLD) that is characterized by inflammation, hepatocyte ballooning and fibrosis. However, the precise cellular processes that trigger the advancement of NASH and the downstream development of HCC remain unclear. Apoptosis, immune cell infiltration, lipotoxicity, de novo lipogenesis, the microbiota and oxidative stress have been shown to activate ER stress, thereby contributing to the initiation of NASH-driven HCC[3]. It is of vital importance to define the cellular processes and pathways that lead to NASH-driven HCC as current treatments for HCC, such as the tyrosine kinase inhibitor sorafenib, are ineffective therapies, as they only extend median survival by a few weeks to months in a subset of patients[4].

This year, three important publications have shed more light on our understanding of NASH-driven HCC by examining the role of caspase 2 in ER-stress-promoted NASH development[5], inhibiting acetyl-CoA carboxylase (ACC) to suppress lipogenesis and HCC[6] and targeting ER stress by pharmacological agents or Bax inhibitor 1 (BI-1) to block IRE1α signalling to explore therapeutic strategies for NASH[7]. In a study by the Karin laboratory, published in 2014, the authors used a fairly new mouse model for NASH-driven HCC, the *MUP-uPA* mouse. This mouse model transiently expresses urokinase in hepatocytes. When maintained on a standard rodent chow diet, the mouse is fairly normal, but feeding it a high-fat diet increases ER stress, such that NASH can be observed by the time they are 16 weeks of age[8]. ER stress induces the expression of caspase 2, a non-apoptotic caspase, which is elevated in *MUP-uPA* mice and in patients with NASH[5]. As hepatocytes with elevated de novo lipogenesis and cholesterol synthesis[9] (both key contributing factors in NAFLD and NASH pathogenesis) are not apoptopic, caspase 2 was investigated as a candidate in the control of sterol regulatory element-binding protein (SREBP)-driven NASH progression in affected patients and *MUP-uPA* mice. Kim and co-workers[5] show that caspase 2 is required for NASH development as *Casp2*$^{-/-}$ *MUP-uPA* mice did not present with fatty liver or raised levels of cholesterol. These mice also exhibit less hepatocyte ballooning and macrophage infiltration than control mice. Furthermore, the animals showed raised levels of AMPK phosphorylation in the liver, muscle and adipocytes.

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This latter observation is consistent with the results of Lally and colleagues[6] showing that therapies aimed at mimicking the inhibitory effect of AMPK phosphorylation on ACC might be an important treatment for NASH-driven HCC. Mice with a loss of AMPK-mediated ACC inhibition show elevated hepatic lipogenesis and early signs of NAFLD and fibrosis, but, even more importantly, when challenged with the carcinogen diethyl nitrosamine (DEN), these mice had twice as many lesions per liver than did wild-type control mice. Treating these animals with the ACC inhibitor ND-654, a molecule that mimics the physiological inhibition of AMPK-induced ACC phosphorylation, led to a reduction of DEN-induced tumour burden by 55%. The authors concluded that therapies designed to work in a similar fashion to AMPK phosphorylation on ACC, such as the small molecule ND-654, might be promising strategies in treating NASH-driven HCC.

Key advances

- Endoplasmic reticulum (ER) stress contributes to hepatocellular carcinoma (HCC) that is driven by nonalcoholic steatohepatitis (NASH)[5–7].
- Caspase 2, a non-apoptotic caspase, is activated by the IRE1α branch of ER stress and controls NASH by cleaving site-1 protease, which activates sterol regulatory element-binding proteins and results in NASH development[5].
- Inhibiting Bax inhibitor 1 increases the activity of the IRE1α signalling branch of the ER stress response and results in NASH, but more importantly the phenotype can be reversed by treating Bax inhibitor 1 null mice with an IRE1α RNase activity inhibitor[7].
- Another approach to treat NASH-driven HCC is the use of small molecules mimicking AMPK-mediated acetyl-CoA carboxylase (ACC) inhibition as ACC-activating mutations increase hepatic carcinogenesis[6].

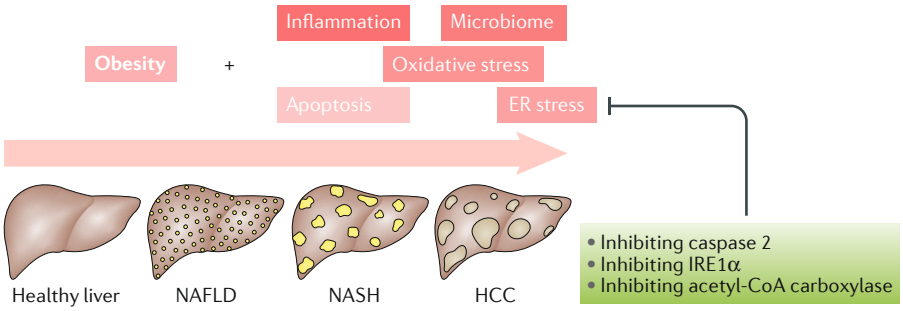

Fig. 1 | **The spectrum of disease progression from a healthy liver to hepatocellular carcinoma due to lipid overload.** ER, endoplasmic reticulum; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

Caspase 2 translation depends on IRE1α signalling, which is one branch of the unfolded protein response of the ER. Inhibiting IRE1α inhibits expression of the caspase 2 protein in the liver without affecting caspase 2 mRNA levels and it prevents SREBP2 activation. Liver biopsy samples obtained from patients with NASH contain increased levels of SREBP2 mRNA, which further points to an important role for caspase 2 in NASH progression[5]. Indeed, direct caspase 2 ablation also abolishes SREBP1 and SREBP2 activation. SREBPs have a key role in NASH development as they regulate cholesterol metabolism in the liver[10]. When a cell is in need of gene products involved in regulating cholesterol synthesis, SREBP precursors are transported from the ER to the Golgi where they will be cleaved by the proteases site-1 protease (S1P) and site-2 protease (S2P). The mature forms of SREBP1 and SREBP2 will be released and will then travel to the nucleus to act as transcription factors. As SREBP1 and SREBP2 lack caspase 2 cleavage sites, Kim at al.[5] investigated how caspase 2 can control SREBP activation. They show that in livers from *Casp2*$^{-/-}$ *MUP-uPA* mice, the levels of membrane-associated full length S1P were increased and no S1P polypeptides were present in the ER, which indicates that caspase 2 activates S1P, thereby promoting SREBP activation followed by the excessive accumulation of free cholesterol that contributes to NASH development.

Caspase 2-generated S1P cleavage products enter the circulation and are found in human patients with NASH at much higher levels than in healthy controls and even patients with NAFLD[5]. This finding might enable the use of caspase 2-generated S1P cleavage products as a much-needed non-invasive biomarker for NASH. As Kim and co-workers[5] clearly showed that caspase 2 inhibition prevented NASH, they investigated the therapeutic value of this approach and injected a caspase 2 inhibitor into *MUP-uPA* mice fed a high-fat diet. They observed that the treatment prevented hepatic steatosis, alongside reduced ballooning of hepatocytes, decreased macrophage recruitment and less fibrosis than in the control group.

> “This approach is a promising strategy for finding new treatments for NASH-driven HCC”

As mentioned above, caspase 2 is directly influenced by IRE1α signalling and therefore ER stress. Targeting and reducing ER stress to control NASH development seems to be a valuable approach, which has also been demonstrated by Lebeaupin and colleagues[7]. In their study, the authors blocked BI-1 (encoded by *Tmbim6*), a negative regulator of IRE1α, to test whether this promoted NASH development, as they observed that BI-1 levels in patients with NAFLD were downregulated and IRE1α levels were upregulated. NASH was observed in *Tmbim6*$^{-/-}$ mice fed a high-fat diet, along with elevated levels of IRE1α, XBP1, CHOP, ALT and AST and activation of the NLRP3 inflammasome, which highlights the importance of inflammation and ER stress in the disease aetiology. More importantly, treating these mice with an IRE1α RNase inhibitor reversed the phenotype and rescued the animals from developing NASH.

Together, these studies from the past year highlight the importance of targeting the ER stress response in NASH-driven HCC (FIG. 1). This approach is a promising strategy for finding new treatments for NASH-driven HCC, a disease that is markedly on the rise in all parts of the globe.

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1. Fu, S., Watkins, S. M. & Hotamisligil, G. S. The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. *Cell Metab.* **15**, 623–634 (2012).
2. Febbraio, M. A. et al. Preclinical models for studying NASH-driven HCC: how useful are they? *Cell Metab.* https://doi.org/10.1016/j.cmet.2018.10.012 (2018).
3. Kanda, T. et al. Apoptosis and non-alcoholic fatty liver diseases. *World J. Gastroenterol.* **24**, 2661–2672 (2018).
4. Ringelhan, M. et al. The immunology of hepatocellular carcinoma. *Nat. Immunol.* **19**, 222–232 (2018).
5. Kim, J. Y. et al. ER stress drives lipogenesis and steatohepatitis via caspase-2 activation of S1P. *Cell* **175**, 133–145 (2018).
6. Lally, J. S. V. et al. Inhibition of acetyl-CoA carboxylase by phosphorylation or the inhibitor ND-654 suppresses lipogenesis and hepatocellular carcinoma. *Cell Metab.* https://doi.org/10.1016/j.cmet.2018.08.020 (2018).
7. Lebeaupin, C. et al. Bax inhibitor-1 protects from nonalcoholic steatohepatitis by limiting inositol-requiring enzyme 1 alpha signaling in mice. *Hepatology* **68**, 515–532 (2018).
8. Nakagawa, H. et al. ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. *Cancer Cell* **26**, 331–343 (2014).
9. Liang, J. Q. et al. Dietary cholesterol promotes steatohepatitis related hepatocellular carcinoma through dysregulated metabolism and calcium signaling. *Nat. Commun.* **9**, 4490 (2018).
10. Brown, M. S. & Goldstein, J. L. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **89**, 331–340 (1997).

Competing interests
The authors declare no competing interests.