EDITORIAL

For reprint orders, please contact: reprints@futuremedicine.com

How endoplasmic reticulum stress contributes to obesity-driven hepatic tumorigenesis

Hayato Nakagawa*

“...endoplasmic reticulum stress is a key link between metabolism and inflammation in obesity-driven hepatocarcinogenesis.”

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. More than 90% of HCCs develop as a result of chronic liver disease, and chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is the main cause in most areas. However, several studies in Western countries showed that 30–40% of patients with HCC do not have chronic HBV or HCV infections [1]. Although this cluster includes alcoholic liver injury and some genetic liver diseases, such as hemochromatosis, other cases are more likely to be associated with obesity and metabolic syndrome and are considered to be based on nonalcoholic steatohepatitis (NASH). Additionally, obesity increased the risk of HCC development in patients with viral hepatitis [2]. Because its prevalence has been increasing worldwide, obesity's potential association with hepatocarcinogenesis has attracted considerable attention in recent years. Although the mechanism by which obesity and metabolic syndrome promote hepatocarcinogenesis is not fully understood, it seems likely to be mediated, in part, by a state of chronic inflammation [3,4]. Importantly, recent evidence revealed that endoplasmic reticulum

(ER) stress is a key link between metabolism and inflammation in obesity-driven hepatocarcinogenesis.

The ER is an important intracellular organelle, responsible for the folding and trafficking of proteins, lipid synthesis and maintenance of calcium homeostasis. Various environmental, physiological and pathological factors, including nutrient fluctuation, can disrupt ER function, which causes protein misfolding and accumulation of misfolded proteins, referred to as ‘ER stress’ [5]. Such disturbances in ER function trigger intracellular signal transduction pathways known as the ‘unfolded protein response’ (UPR). The UPR comprises three main signaling branches, originating from ER transmembrane proteins: PERK, ATF6 and IRE1 α . These sensor proteins remain inactive as long as they are bound to the intraluminal chaperone glucose-regulated protein 78 (GRP78)/Bip. However, under ER stress, GRP78 binds preferentially to unfolded proteins, dissociating from PERK, ATF6 and IRE1 α , and leading to their activation. The UPR initially aims to restore homeostasis to allow the cell to adapt to the stressor(s) by translational inhibition and

Hepatic Oncology

KEYWORDS

- endoplasmic reticulum stress
- hepatocellular carcinoma
- nonalcoholic steatohepatitis
- obesity

“...endoplasmic reticulum stress and hypernutrition cooperatively advance liver disease from ‘simple’ steatosis to nonalcoholic steatohepatitis...”

*Department of Gastroenterology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655; Tel.: +81 3 3815 5411; Fax: +81 3 3814 0021; hanakagawa-tyk@umin.ac.jp

ER chaperone upregulation. However, chronic or unresolved ER stress triggers apoptosis via several mechanisms, such as CHOP-, JNK- and caspase-dependent pathways [6].

Disruption of ER homeostasis and UPR activation has been linked to lipid biosynthesis, insulin resistance, inflammation and apoptosis, all of which are important components of NASH. Especially, ER stress-induced liver steatosis has been one of the most extensively studied issues. Several mechanisms of ER stress-induced liver steatosis have been proposed: decreased liver lipid export, due to suppression of apolipoprotein B expression by UPR-mediated translational attenuation [7], increased *de novo* lipogenesis by ER stress-induced SREBP activation [8], XBP1-mediated induction of lipogenic enzymes [9], decreased lipid secretion by IRE1-dependent mRNA decay (RIDD) [10] and insulin resistance, caused by ER stress-induced JNK and IKK activation [11]. At the same time, steatosis, hypernutrition, obesity and insulin resistance, in turn, promote ER stress. Thus, ER stress and liver steatosis can form a vicious cycle [12]. Although its physiological role has not been examined until recently, we demonstrated, using a new mouse model, that ER stress and hypernutrition cooperatively advance liver disease from 'simple' steatosis to NASH, subsequently leading to spontaneous HCC development [13].

We used *MUP-uPA* transgenic mice to investigate ER stress in the liver [14]. *MUP-uPA* mice express the uPA protein in mature hepatocytes, where it accumulates in the ER, thereby leading to chronic ER stress in hepatocytes. Remarkably, feeding a high-fat diet (HFD) to *MUP-uPA* mice results in steatohepatitis that closely resembles the pathology of human NASH, with ballooning degeneration, hepatocyte death and pericellular/bridging fibrosis, eventually leading to spontaneous HCC development. The major NASH-promoting effects of ER stress in this system were increased lipogenesis by SREBP activation, oxidative stress and susceptibility to lipotoxic cell death. Although the mechanism of ER stress-induced SREBP activation has not been fully determined, recent evidence has resulted in three proposed mechanisms: GRP78 dissociation from the SCAP-SREBP complex, eIF2 α -dependent downregulation of INSIG and caspase-induced SREBP cleavage [15]. In fact, adenoviral delivery of GRP78 to the *MUP-uPA* hepatocytes suppressed SREBP activation. A recent study demonstrated that

chronic hepatic ER stress, caused by obesity, increased ER-mitochondria contact, which resulted in mitochondrial dysfunction and oxidative stress [16]. Thus, the vicious cycle of ER stress and steatosis via SREBP activation synergistically increases reactive oxygen species production in hepatocytes, causing oxidative stress and its sequelae, including genomic instability, oncogenic mutations and/or gene copy number changes. Additionally, ER and oxidative stress increase the susceptibility of hepatocytes to lipotoxic cell death, thereby releasing inflammatory mediators that attract and activate monocytes/macrophages. TNF and other mediators produced by activated inflammatory macrophages stimulate compensatory hepatocyte proliferation and expand HCC progenitors. Moreover, TNF further accelerates liver steatosis and the inflammatory tumor microenvironment in an NF- κ B-dependent manner. Thus, ER stress stands at the crossroads of inflammation and metabolism in HCC development via a vicious cycle involving hepatosteatosis [17]. A dilated ER, a sign of ER stress, is seen frequently in human ballooning hepatocytes [18]. Administration of the ER stress inducer tunicamycin to HFD-fed wild-type mice also rapidly induced ballooning degeneration and hepatocyte death [13]. These findings suggest that excess ER stress may be a causal factor of hepatocyte ballooning, a key diagnostic and prognostic feature in NASH. Furthermore, HBV and HCV infection also induce ER stress [6], and liver steatosis has been reported to be an independent risk factor for HCC development [19]. Thus, the vicious cycle of ER stress and hypernutrition may also accelerate hepatocarcinogenesis in chronic viral hepatitis patients with obesity.

Interrupting this cycle could be a promising therapeutic target for NASH and HCC. In fact, administration of the chemical chaperones, 4-phenylbutyrate and tauroursodeoxycholic acid and adenovirus-mediated hepatic GRP78 overexpression reduced ER stress and improved liver pathology significantly in HFD-fed *MUP-uPA* mice. We also investigated the therapeutic potential of UPR inhibition in the treatment of NASH and HCC, focusing on CHOP, because CHOP, downstream of PERK, has been considered to play an important role in ER stress-induced cell death [6]. However, liver-specific ablation of CHOP in HFD-fed *MUP-uPA* mice did not improve NASH severity and rather enhanced tumor multiplicity, due to decreased apoptosis

“Reducing endoplasmic reticulum stress may be a promising therapeutic strategy...”

of initiated hepatocytes. Because an appropriate UPR is an adaptive and protective response to ER stress, simple inhibition of the signaling pathway might not be effective, but harmful. Further studies are needed to clarify the implications and therapeutic potential of UPR in NASH and HCC. However, ablation of the TNF receptor 1 or the TNF antagonist etanercept can suppress tumor development. Thus, anti-TNF drugs in combination with improved intrahepatic delivery of chemical chaperones to reduce ER stress may be promising treatment options for NASH and obesity-mediated HCC development.

In conclusion, ER stress in hepatocytes combined with hypernutrition results in a NASH-like disease that spontaneously progresses to HCC thorough an inflammatory mechanism dependent on TNF and IKK signaling.

Reducing ER stress may be a promising therapeutic strategy to prevent obesity-driven hepatic tumorigenesis. However, the role of UPR in hepatic tumorigenesis remains poorly understood. Further investigations may allow the future design of effective therapies to prevent and treat NASH/obesity-mediated HCC.

Financial & competing interests disclosure

This work was supported by grants from the Japanese Society of Gastroenterology, The Tokyo Society of Medical Sciences and Kanae Foundation for the Promotion of Medical Science. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

- El-Serag HB. Hepatocellular carcinoma. *N. Engl. J. Med.* 365, 1118–1127 (2011).
- Chen CL, Yang HI, Yang WS *et al.* Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. *Gastroenterology* 135, 111–121 (2008).
- Park EJ, Lee JH, Yu GY *et al.* Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 140, 197–208 (2010).
- Nakagawa H, Maeda S. Inflammation- and stress-related signaling pathways in hepatocarcinogenesis. *World J. Gastroenterol.* 18, 4071–4081 (2012).
- Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. *Nat. Rev. Cancer* 14, 581–597 (2014).
- Malhi H, Kaufman RJ. Endoplasmic reticulum stress in liver disease. *J. Hepatol.* 54, 795–809 (2011).
- Ota T, Gayet C, Ginsberg HN. Inhibition of apolipoprotein B100 secretion by lipid-induced hepatic endoplasmic reticulum stress in rodents. *J. Clin. Invest.* 118, 316–332 (2008).
- Kammoun HL, Chabanon H, Hainault I *et al.* GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. *J. Clin. Invest.* 119, 1201–1215 (2009).
- Lee AH, Scapa EF, Cohen DE *et al.* Regulation of hepatic lipogenesis by the transcription factor XBP1. *Science* 320, 1492–1496 (2008).
- So JS, Hur KY, Tarrio M *et al.* Silencing of lipid metabolism genes through IRE1 α -mediated mRNA decay lowers plasma lipids in mice. *Cell Metab.* 16, 487–499 (2012).
- Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 140, 900–917 (2010).
- Dara L, Ji C, Kaplowitz N. The contribution of endoplasmic reticulum stress to liver diseases. *Hepatology* 53, 1752–1763 (2011).
- Nakagawa H, Umemura A, Taniguchi K *et al.* ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. *Cancer Cell* 26, 331–343 (2014).
- Weglarz TC, Degen JL, Sandgren EP. Hepatocyte transplantation into diseased mouse liver. Kinetics of parenchymal repopulation and identification of the proliferative capacity of tetraploid and octaploid hepatocytes. *Am. J. Pathol.* 157, 1963–1974 (2000).
- Colgan SM, Hashimi AA, Austin RC. Endoplasmic reticulum stress and lipid dysregulation. *Expert Rev. Mol. Med.* 13, e4 (2011).
- Arruda AP, Pers BM, Parlakgul G *et al.* Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat. Med.* 20, 1427–1435 (2014).
- Maurel M, Samali A, Chevet E. Endoplasmic reticulum stress: at the crossroads of inflammation and metabolism in hepatocellular carcinoma development. *Cancer Cell* 26, 301–303 (2014).
- Caldwell S, Ikura Y, Dias D *et al.* Hepatocellular ballooning in NASH. *J. Hepatol.* 53, 719–723 (2010).
- Ohata K, Hamasaki K, Toriyama K *et al.* Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 97, 3036–3043 (2003).