Endoplasmic Reticulum Stress: At the Crossroads of Inflammation and Metabolism in Hepatocellular Carcinoma Development

Marion Maurel,[1,4] Afshin Samali,[1] and Eric Chevet[2,3,4,*]
[1]Apoptosis Research Centre, School of Natural Sciences, NUI Galway, Galway, Ireland
[2]Inserm U1053, 33000 Bordeaux, France
[3]University of Bordeaux, 33000 Bordeaux, France
[4]Centre de Lutte Contre le Cancer Eugène Marquis, 35000 Rennes, France
*Correspondence: eric.chevet@inserm.fr
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Steatohepatitis is a cause of hepatocellular carcinoma development; however, the underlying mechanisms are poorly defined. In this issue of *Cancer Cell*, Nakagawa and colleagues demonstrate that activation of endoplasmic reticulum stress signaling is instrumental in the development of steatohepatitis and synergizes with proinflammatory pathways to promote hepatocarcinogenesis.

Over the past two decades, the rising incidence of hepatocellular carcinoma (HCC) has paralleled an increased prevalence of obesity, suggesting that the two may be linked. Indeed, obesity fuels the production of proinflammatory cytokines leading to the accumulation of free fatty acids in hepatocytes, a condition termed steatohepatitis (Starley et al., 2010). This pathology leads to chronic inflammation which, in turn, induces nonalcoholic steatohepatitis (NASH), a risk factor in the promotion of HCC. Activation of endoplasmic reticulum (ER) stress has been clearly shown to contribute to liver steatosis, steatohepatitis, and NASH (Starley et al., 2010). However, the role of ER stress signaling in the development and progression of HCC is much less documented. Recent reports linking the unfolded protein response (UPR) to inflammation (Garg et al., 2012) suggest a tightly interconnected network that could certainly be involved in liver carcinogenesis. In this issue of *Cancer Cell*, Nakagawa et al. (2014) report a novel mechanism of carcinogenesis in which the activation of ER stress signals plays a synergistic role with high fat diet (HFD)-induced steatohepatitis to promote the development of HCC (Figure 1).

Nakagawa et al. (2014) utilized the major urinary protein-urokinase plasminogen activator (*MUP-uPA*) transgenic mouse model to study ER stress in hepatocytes. The *MUP-uPA* transgene induces overexpression of the uPA protein, which accumulates in the hepatocyte ER, thereby leading to ER stress and liver lesions in mice. Remarkably, the *MUP-uPA* mice fed with a HFD exhibited greater liver damage, immune infiltration, and increased lipogenesis compared to their control low fat diet counterparts. *MUP-uPA* mice on HFD rapidly displayed pathology indicative of NASH that evolved into HCC over time. It has been reported that the major NASH-promoting effects of ER stress increase lipogenesis through SREBP activation (Kammoun et al., 2009), oxidative stress, and susceptibility to lipotoxic cell death. Moreover, Nakagawa et al. (2014) indicate that in normal hepatocytes both steatohepatitis and HCC development are independent of CHOP, thereby ruling out the involvement of this apoptosis promoting transcription factor in *MUP-uPA* and HFD-induced hepatocyte cell death. Interestingly, the role of ATF6, a major ER stress activated transcription factor likely to be involved in HCC development (Shuda et al., 2003), remains to be investigated in the authors' experimental model.

HCC takes several decades to appear and evolves from premalignant lesions in chronically damaged livers that create the bedding for HCC progenitor cells (HcPCs). The ability of HcPCs to progress into HCC depends on autocrine interleukin 6 production. To investigate the involvement of tumor necrosis factor (TNF) signaling in hepatocarcinogenesis, Nakagawa et al. (2014) transplanted HcPCs from diethylnitrosamine-treated wild-type, *Tnfr1*$^{-/-}$, or *Ikbkb*$^{\Delta hep}$ into MUP-uPA mice. They found tumor growth to be abrogated in HFD-fed mice transplanted with either *Tnfr1*$^{-/-}$ or *Ikbkb*$^{\Delta hep}$ HcPCs compared to those transplanted with wild-type HcPCs, thus demonstrating the major role of TNF and IκB kinase β signaling in hepatocarcinogenesis in HFD-fed mice.

The contribution of ER stress to HCC has been proposed repeatedly in the context of sensitivity to the chemotherapeutic agent sorafenib (Yi et al., 2012), its involvement in hepatocarcinogenesis (Shuda et al., 2003), or, more recently, the presence of somatic mutations in genes coding for components of the UPR or the ER homeostasis control machinery (Guichard et al., 2012). However, thus far, no connection has been made between ER stress signaling and steatohepatitis-induced HCC. The work of Nakagawa et al. (2014) suggests that several mechanisms related to ER stress and hypernutrition could cooperate to induce HCC development. These potential mechanisms could occur through: (1) HFD-induced hepatosteatosis, resulting in mild ER stress in *MUP-uPA* mice due to uPA expression; (2) ER stress-induced SREBP1 activation in *MUP-uPA* mice, thereby enhancing lipogenesis and increasing the degree of hepatic steatosis beyond that achieved by HFD alone; (3) increased reactive oxygen species production by ER stress and steatosis in hepatocytes as well as subsequent oxidative stress and its genotoxic consequences (Figure 1); (4) ER and oxidative stress-mediated increase in hepatocytes

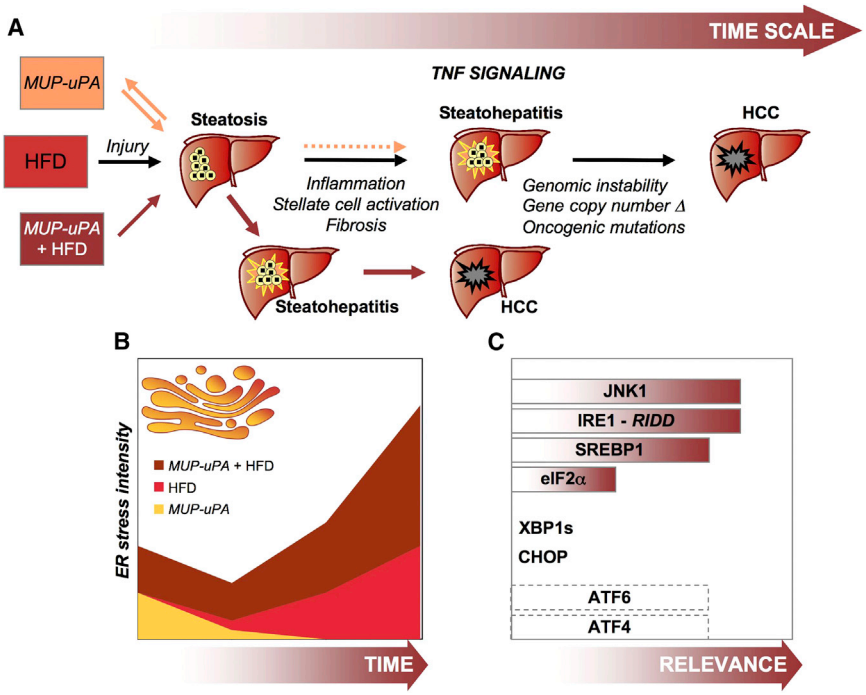

Figure 1. ER Stress in Steatohepatitis-Induced Hepatocarcinogenesis

(A) Schematic timeline of HCC progression as reported by Nakagawa et al. (2014). *MUP-uPA* mice, although exhibiting ER stress in the liver, did not develop HCC, whereas signs of steatosis and weak fibrosis can be observed. On HFD, C57BL/6 mice developed steatosis, steatohepatitis, and HCC (in a TNFR1-dependent manner) over a period of 40 weeks. Finally, HFD applied to *MUP-uPA* mice led to a more penetrant HCC phenotype than in wild-type animals, thus demonstrating the synergistic impact of combined ER stress and HFD on HCC development.

(B) Qualitative representation of the intensity of ER stress observed in the models studied by Nakagawa et al. (2014). *MUP-uPA* mice presented a strong basal ER stress, which dissipated over time with hepatocyte renewal. In contrast, HFD-induced ER stress increases with time. Finally, HFD in *MUP-uPA* mice led to prolonged and reinforced ER stress throughout the experimental pipeline, thus correlating ER stress intensity with HCC outcome.

(C) Qualitative representation of the relevance of ER stress signaling components toward HCC development upon HFD. The IRE1 arm of the UPR appears to play a significant role in steatosis-induced HCC, because the phosphorylation of IRE1 and the activation of JNK are increased and 25% of RIDD targets impact lipid metabolism (although RIDD was not investigated by Nakagawa et al., 2014). The latter is also a direct target of TNF signaling through TNFR1 and thus represents a point of convergence of two of the signaling pathways involved in hepatocarcinogenesis. The activation of SREBP1 also represents an important factor activated upon ER stress that stimulates lipogenesis and enhances the steatotic/steatohepatitic phenotype. Interestingly, the phosphorylation of eIF2α is also observed and relevant, but it might also be due to the activation of the integrated stress response, a key player in metabolic syndrome. Finally, both CHOP and XBP1s appear dispensable for steatohepatitis-induced HCC development. The respective roles of ATF4 and ATF6 remain to be investigated (dotted bars).

sensitivity to lipotoxicity and cell death, thereby releasing inflammatory mediators that attract and activate immune cells; (5) the production of TNF and other mediators by activated inflammatory macrophages, which stimulate compensatory hepatocyte proliferation and expand HCC progenitors; and/or (6) a global change in ER stress activation kinetics and intensities, which could lead to hepatocyte transformation via poorly defined mechanisms (Figure 1).

HCC is extremely difficult to treat and is the third leading cause of deaths associated with cancers worldwide. The current treatments are liver resection and liver transplantation, but few HCC patients can benefit from surgery because most are diagnosed at late stages. Sorafenib is currently the only drug available for HCC and increases survival time by approximately 3 months. In their article, Nakagawa et al. (2014) discuss the possibility of therapeutic intervention through the combined action of anti-TNF therapy and chemical chaperones. As such, the use of chemical chaperones (4-phenyl butyrate or tauroursodeoxycholic acid) has been applied to animal models to resolve liver steatosis and steatohepatitis (Ben Mosbah et al., 2010). This might indeed be efficient for decreasing the steatohepatitis burden, which would in turn be anticipated to reduce the occurrence of HCC. However, the window in which this type of treatment should be applied is still questionable. Indeed, the steatohepatitis/NASH status is not easily diagnosed to allow preventive HCC treatment. Later, at the HCC stage, the use of anti-TNF therapy could reduce the inflammation of the tumor bed. However, one might expect that chemical chaperones may not be the best therapeutic option for tumor cells, because they might actually increase proteostasis and confer proliferative advantages. Alternatively, one could combine anti-TNF therapy and inhibitors of ER stress downstream of the UPR (Hetz et al., 2013). Although, Nakagawa et al. (2014) have shown that XBP1 is not instrumental in NASH development in *MUP-uPA* mice, approximately a quarter of the validated regulated IRE1-dependent mRNA decay of RNA (RIDD) substrates are associated with lipid metabolism (Maurel et al., 2014). Therefore, it would be interesting to investigate the activation status of RIDD in the target hepatocytes because such molecules, and in particular IRE1 inhibitors, were shown to be potentially highly relevant anticancer molecules. Their use would not only be expected to reduce hepatocyte damage, inflammation, and steatosis, but would also slow down tumor growth, because these molecules may exert effects on both the tumor and adjacent parenchyma.

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*FAL1*ing inside an Amplicon

Alejandro Athie[1] and Maite Huarte[1,*]
[1]Center for Applied Medical Research, University of Navarra, 331008 Pamplona, Spain
*Correspondence: maitehuarte@unav.es
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Frequently amplified regions of the cancer genome contain well-known oncogenes. In this issue of *Cancer Cell*, Hu and colleagues discover that *FAL1*, a long noncoding RNA is encoded in one of these regions. *FAL1* acts as an oncogene by stabilizing BMI1, which results in the repression of *CDKN1A* expression.

Genomic instability in cancer leads to rearrangements, amplifications, and deletions of entire DNA fragments. Somatic copy-number alterations (SCNAs) provide a mechanism for modifying the gene dose to confer selective advantage for tumor cells. The amplification of a gene can lead to its overexpression: well-known oncogenes such as *EGFR*, *ERBB2*, and *MYC* are contained in frequently-amplified regions (Beroukhim et al., 2010).

Amplicon length can vary from several kilobases to megabases, often containing many genes. A big challenge for cancer biology is to identify which of the genes contained in an amplified region play a causal role in carcinogenesis. The challenge is even greater if we take a closer look and consider not only the protein-coding genes but also the long noncoding RNAs (lncRNAs) present in these amplified regions. Although growing evidence relates some lncRNAs with cancer progression, the function of the vast majority of lncRNAs remains to be determined, complicating the task.

In this issue of *Cancer Cell*, Hu et al. (2014) rise to this challenge by focusing their study on the noncoding portion of the genome to identify lncRNAs that are clinically relevant to cancer. They show that a large number of lncRNAs are inside somatic copy number alterations. Some of these altered regions do not contain any previously identified cancer-associated protein-coding genes, suggesting that the noncoding genes could be responsible for driving the disease. These analyses reveal a set of noncoding candidate cancer drivers and highlight the potential role of lncRNAs in the development of cancer.

By integrating SNP arrays of 2,394 tumors of 12 cancer types with gene expression microarrays of 40 cancer cell lines, Hu et al. (2014) identify a set of expressed lncRNAs frequently amplified in tumors in the study. From this set, the authors explore in depth the role of a novel lncRNA and putative oncogene named *f*ocally *a*mplified *l*ncRNA on chromosome *1* or *FAL1*.

More detailed analysis of *FAL1* copy number gain showed that this alteration was frequently present in epithelial tumors. Interestingly, the high level of *FAL1* expression is not always associated with its focal amplification, suggesting that other mechanisms may contribute to its increased expression in cancer cells. Further analysis using an ovarian cancer tumor cohort revealed a higher expression of *FAL1* in late-stage tumors and an association between the genomic amplification of *FAL1* and decreased patient survival.

Besides the strong genetic evidence provided by the SCNA analysis, *FAL1* displayed oncogenic features in several functional experiments. Overexpression of *FAL1* resulted in an increase in the colony-formation capacity of cells, an effect enhanced by the additional overexpression of *MYC* or mutant *RAS*. These experiments not only indicate that *FAL1* can act in cooperation with other oncogenes, but also suggest that the lncRNA exerts its functions *in trans.* In fact, downregulation of *FAL1* by short hairpin RNAs showed no effect on the expression levels of other genes present in the amplicon. Interestingly, among these genes is *MCL1*, a known protein-coding oncogene (Beroukhim et al., 2010). However, alteration of *FAL1* levels had no effect on *MCL1*, suggesting an independent role. Similar to *FAL1*, the oncogenic lncRNA *PCAT-1* has been recently shown to appear coamplified with an oncogene (MYC in this case), while it functions independently of its neighboring oncogene, i.e., via a MYC-independent mechanism (Prensner et al., 2011). In contrast, a recent study (Tseng