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KEAP the balance between life and death

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Hepatocyte apoptosis in association with oxidative stress represent key pathogenic factors involved in tumor development in patients with non-alcoholic fatty liver disease (NAFLD). In our recent study, we established that cellular degradation of Kelch-like ECH-associated protein 1 (KEAP1) through sequestrosome (SQSTM)1/p62-dependent autophagy activates c-Jun NH2 terminal kinase (JNK), upregulates expression of Bcl-2–interacting mediator (BIM) and p53 upregulated modulator of apoptosis (PUMA), and contributes to hepatocyte apoptosis induced by saturated free fatty acids. These findings raise the possibility that dysregulation of KEAP1 may contribute to liver cell death and tumorigenesis during chronic inflammatory liver disease.

The Kelch-like ECH-associated protein 1 (KEAP1)/ Nuclear factor (erythroid-derived 2)-like 2 (NRF2) pathway is a major sensor of oxidative stress and its activation is widely considered to be an adaptive response to protect cells from injury. KEAP1 serves as an adaptor for ubiquitin E3 ligase and promotes proteasomal degradation of NRF2, a transcription factor that regulates the expression of various antioxidant enzymes. Under oxidative/electrophilic stress conditions, KEAP1 is inactivated resulting in NRF2 stabilization and the activation of a protective gene expression program.[1] Thus, low or high NRF2 protein levels can either sensitize or protect, respectively, against oxidative stress-dependent cell death. Although genetic deficiency in *Keap1* protects hepatocytes against drug-induced toxicity,[1] a recent study identified that genetic ablation of *Keap1* compromises cell viability by interfering with the cellular ability to clear protein aggregates,[2] thus revealing an unexpected positive function of KEAP1 in regulating cellular viability. Saturated free fatty acid (sFFA)-induced hepatocyte apoptosis (or lipoapoptosis) and oxidative stress represent key pathophysiological mechanisms in non-alcoholic fatty liver disease (NAFLD) pathogenesis.[3] Therefore, in our recent study we evaluated the role of KEAP1 in hepatocyte lipotoxicity.[4] In both primary and transformed liver cells, we observed that sFFA-induced toxicity was associated with degradation of KEAP1, in part through autophagy in a sequestrosome (SQSTM)1/p62-dependent manner. We also identified that loss of *Keap1* induced spontaneous liver cell death and further increased hepatocyte susceptibility to a lipotoxic insult. This effect was associated with increased activation of c-Jun NH2 terminal kinase (JNK) in an NRF2-independent manner and upregulation of protein levels of Bcl-2–interacting mediator (BIM) and p53 upregulated modulator of apoptosis (PUMA), 2 proapoptotic BH3-only proteins that induce hepatocyte cell death.[4]

Autophagy is often observed in dying cells as it is activated by oxidative stress. Although it is mostly considered a prosurvival pathway it can also contribute to cell death, especially if the process relates to lysosomal dysfunction and the activation of caspase-8, which are both implicated in lipotoxicity.[3] In our model, sFFAs induced hepatocyte apoptosis but also stimulated the formation of autophagic vacuoles in liver cells. Interestingly, knockdown of the autophagy substrate *p62* decreased sFFA-induced KEAP1 degradation and maintaining KEAP1 protein levels upon lipotoxic insult decreased sFFA-induced toxicity. However, a recent study suggests that activated autophagy plays a protective role against sFFA-induced apoptosis in hepatocytes.[5] Thus, it is possible that autophagy may first represent an attempt of the cell to adapt to stress to induce antioxidant responses, but that prolonged autophagy-induced degradation of KEAP1 is subsequently detrimental to the cell by activating prodeath signals and by inhibiting clearance of protein aggregates,[2] supporting the idea of a "double edged" role of autophagy in determining cellular fate. In our study, we found that the positive function of KEAP1 in regulating hepatocyte susceptibility to lipoapoptosis relied on its ability to repress sFFA-induced JNK activation and downstream upregulation of the proapoptotic proteins BIM and PUMA. Although the mechanisms by which KEAP1 exerts its inhibitory effect on JNK activity are not completely clear, JNK can be added, together with IkB kinase β (IKKβ),[6] to the list of kinases regulated by KEAP1. Thus, KEAP1 induction could potentially be leveraged to prevent JNK-dependent cellular toxicity.

KEAP1 might play a pertinent role in the pathogenesis of NAFLD, a disease associated with obesity, insulin resistance, hepatic steatosis, elevated levels of circulating FFAs, dysregulated autophagy, and increased JNK activation, PUMA expression, and hepatocyte apoptosis.[3] Intriguingly, *Keap1* deletion worsened insulin resistance (a physiological response associated with excessive JNK1 activity) and increased hepatocyte steatosis in diet-

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induced and genetic obesity in mice[1,4] and additional studies will be necessary to determine whether silencing of *Keap1* also increases high-fat diet-induced hepatocyte injury. Insulin resistance, in association with hepatocyte lipotoxicity and liver inflammation, coincides with a higher risk of developing liver cancers in the onset of NAFLD; therefore, understanding whether dysregulation of KEAP1-dependent signaling pathways represents a new cellular and molecular mechanism that accounts for this relationship is germane to this disease and remains to be verified in appropriately designed studies.

Liver cancer (hepatocellular carcinoma, or HCC) often arises in a setting of chronic hepatic inflammation. During this process, excessive hepatocyte damage caused by the continued expression of proinflammatory cytokines and recruitment of activated immune cells to the liver is accompanied by compensatory hepatocyte proliferation, which provides a mitogenic and mutagenic environment favorable for the development of HCC.[7] HCC is a genetically heterogeneous disease, and loss-of-function mutations in *KEAP1* have been recently identified in multiple cohorts of HCC.[8] Interestingly, new evidence suggests that JNK1 plays an important role in tumor initiation and progression in HCC, as higher activation of JNK1 was measured in HCC samples and was associated with poorer prognosis in patients.[9] Also, JNK1/PUMA-dependent apoptosis was identified as a key molecular mechanism in the development of chemical-induced hepatocarcinogenesis.[10] Whether a direct negative correlation between KEAP1 protein levels and JNK1 activity accounts for hepatocyte cell death and compensatory proliferation in HCC remains to be investigated.

In conclusion, we identified that degradation of KEAP1 through p62-dependent autophagy contributes to lipotoxicity by activating the proapoptotic BH3-only proteins BIM and PUMA via JNK. Given the strong association between hepatocyte apoptosis and cancer development during inflammatory chronic liver disease, it will be interesting to understand whether dysregulation of signaling cascades involving KEAP1 represents an additional link between apoptosis, inflammation, and cancer pathogenesis. Further investigations will be needed to ascertain whether induction of KEAP1 has clinical implications for the treatment or prevention of JNK-mediated liver injury.

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