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Induction of Autophagy, a Promising Approach for Treating Liver Injury

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Abstract

Recent investigations have demonstrated a complex interrelationship between autophagy and cell death. A common mechanism of cell death in liver injury is tumor necrosis factor (TNF) cytotoxicity. To better delineate the *in vivo* function of autophagy in cell death, we examined the role of autophagy in TNF-induced hepatic injury. Atg7^{-/-} hep mice with a hepatocyte-specific knockout of the autophagy gene *atg7* were generated and cotreated with D-galactosamine (GalN) and lipopolysaccharide (LPS). GalN/LPS-treated Atg7^{-/-} hep mice had increased serum alanine aminotransferase levels, histological injury, numbers of TUNEL (terminal deoxynucleotide transferase-mediated deoxyuridine triphosphate nick end-labeling)-positive cells and mortality as compared with littermate controls. Loss of hepatocyte autophagy similarly sensitized to GalN/TNF liver injury. GalN/LPS injury in knockout animals did not result from altered production of TNF or other cytokines. Atg7^{-/-} hep mice had accelerated activation of the mitochondrial death pathway and caspase-3 and -7 cleavage. Increased cell death did not occur from direct mitochondrial toxicity or a lack of mitophagy, but rather from increased activation of initiator caspase-8 causing Bid cleavage. GalN blocked LPS induction of hepatic autophagy, and increased autophagy from beclin 1 overexpression prevented GalN/LPS injury. Autophagy, therefore, mediates cellular resistance to TNF toxicity *in vivo* by blocking activation of caspase-8 and the mitochondrial death pathway, suggesting that autophagy is a therapeutic target in TNF-dependent tissue injury.

Comment

Autophagy is a catabolic lysosomal degradation process, which was first discovered in the liver by de Duve and Wattianux about 50 years ago. Currently, over 30 AuTophagy-related (ATG) genes have been identified, which regulate the process of autophagy through multiple signaling pathways.¹ Increasing evidence now supports cross talk between autophagy and cell death. In response to stress, cells are able to survive by utilizing autophagy as a survival mechanism by generating nutrients during starvation, eliminating invading pathogens as well as removing damaged proteins and organelles. In the liver, basal autophagy seems to be critical to maintain liver homeostasis and cell survival because genetic deletion of Atg5 or Atg7 in the mouse liver results in increased cell death and severe hepatomegaly and liver injury.^{2, 3}

In the liver, a common cell death pathway resulting in liver injury is mediated by tumor necrosis factor- α (TNF- α). Co-injection of mice with lipopolysaccharide (LPS) and D-galactosamine (GalN) is a well-established animal model to study TNF- α -induced hepatocyte apoptosis and liver injury.⁴ Induction of apoptosis is mediated by two pathways, the extrinsic and intrinsic pathways.⁵ The extrinsic pathway is initiated by the binding of extracellular death ligands such as TNF- α or Fas ligand with their respective death receptors on the cell surface. Ligation of the death receptors activates the assembly of the death-inducing signaling complex (DISC), which activates caspase-8 that further cleaves downstream effector caspases (caspase-3, 6, 7) to trigger apoptosis in certain cell types (Type I). However, in Type II cells such as hepatocytes, activation of the extrinsic pathway alone is not sufficient to induce apoptosis. Activated caspase-8 cleaves Bid and the resultant truncated Bid (tBid) translocates to mitochondria to activate the mitochondrial death pathway which further amplifies the apoptotic signals to induce apoptosis.^{5, 6}

Currently it is not clear how autophagy affects death-receptor-induced apoptosis in the liver or other organs. Mice with the global deletion of either Atg7 or Atg5 die shortly after birth, and liver-specific deletion of Atg7 or Atg5 by a constitutively expressed albumin-Cre in mice also leads to severe liver injury as well as compensatory activation of the anti-oxidant transcription factor Nrf-2.^{3, 7} To overcome the disadvantages of these mouse models, Amir et al. utilized novel inducible hepatocyte-specific Atg7 knockout (Atg7^{hep}) mice that were challenged with LPS/D-GalN to investigate the complex role of autophagy and cell death in the liver.⁸ In this model, Atg7^{flox/flox} mice were crossed with tamoxifen-inducible, albumin promoter-driven Cre mice in which Atg7 was deleted in adult mice by injection with tamoxifen. Atg7^{hep} mice had no sign of liver injury for 2 weeks following tamoxifen injection. Interestingly, they found that in response to LPS/D-GalN Atg7^{hep} mice had increased serum alanine aminotransferase levels, histological injury and mortality when compared with littermate wild type control mice. There were no differences in the levels of cytokines and TNF receptor 1 between Atg7^{hep} and wild type controls. Autophagy has been shown to protect against drug- and alcohol-induced liver injury by selective removal of damaged mitochondria (mitophagy).^{9, 10} However, no changes in the mitochondrial DNA content or levels of cytochrome c oxidase (an inner mitochondrial membrane protein) were found in either wild type or Atg7^{hep} mice following LPS/D-GalN treatment. Furthermore, they also found similar levels of cellular ATP and oxidative stress in LPS/D-GalN-treated wild type and Atg7^{hep} mice. LPS/D-GalN-treated Atg7^{hep} mice had increased JNK and caspase-8 activation resulting in enhanced Bid cleavage and subsequent increased mitochondrial cytochrome c release and caspase-3/7 activation as compared to wild type controls.⁸

Perhaps one of the most intriguing findings was that the lack of autophagy in mouse liver promotes LPS/D-GalN-induced caspase-8 activation. Upon TNF- α binding with TNFR1, the cellular FLICE-like protein (c-FLIP) competes with caspase-8 for binding with FADD and inhibits caspase-8 activation. Amir et al. found that loss of hepatocyte autophagy decreased the levels of c-FLIP_L and thus promoted caspase-8 activation in LPS/D-GalN-treated Atg7^{deltahep} mice.⁸ Although it was not clear whether the decreased c-FLIP_L was due to the decreased transcription or posttranslational regulation, Amir et al. suggested that increased JNK activation in the Atg7^{hep} mice by LPS/D-GalN could account for the

decreased c-FLIP_L. However, it is possible that other mechanisms might also be involved in the increased caspase-8 activation in Atg7^{-/-} hep mice in addition to the decreased c-FLIP_L. First, SQSTM1/p62 (hereafter referred to as p62), a substrate protein which is usually degraded by autophagy, promotes the aggregation of CUL3 ubiquitin ligase-modified caspase-8 on the p62 positive speckles which allows the full activation of caspase-8¹¹. Because autophagy-deficient mouse livers have accumulation of aberrant p62, it is possible that increased p62 levels in the Atg7^{-/-} hep mice might also promote caspase-8 activation following LPS/D-GalN treatment. Second, autophagy can also suppress cell death by autophagic degradation of active caspase-8 in TRAIL-treated cancer cells.¹² Third, it has been reported that caspase-8 can be recruited to the p62 and LC3-II positive autophagosome membranes to facilitate caspase-8 self-cleavage resulting in apoptosis in a p62- and LC3-dependent manner in cells treated with a proteasome inhibitor or a pan-sphingosine kinase inhibitor^{13, 14}. However, it seems less likely that LPS/D-GalN-induced caspase-8 activation in Atg7^{-/-} hep mice would also involve the association of caspase-8 on the autophagosome membranes because there is no autophagosome membrane formation in the absence of Atg7.²

Another intriguing finding in the studies by Amir et al. was that LPS treatment alone induced autophagic flux in the mouse liver, which was suppressed by the GalN.⁸ Interestingly, Amir et al. did not find significant changes in the protein levels of several key autophagy proteins that they assessed. These results seemed to be surprising because one would assume a transcriptional regulation of autophagy genes might be involved since GalN inhibits the general transcription in the liver. It has been reported that transcription factor EB (TFEB) plays a critical role in regulating autophagy in hepatocytes.¹⁵ However, LPS treatment increased adenosine monophosphate-activated protein kinase (AMPK) in the mouse liver, which was inhibited by GalN.⁸ Recent evidence indicates that AMPK activates autophagy by multiple different mechanisms. AMPK can inactivate mammalian target of rapamycin (mTOR), a key negative regulator of autophagy, through direct phosphorylation of TSC2 to induce autophagy. However, this mechanism seems less likely to be involved in LPS-induced autophagy because mTOR was not altered by LPS treatment in the mouse liver. Moreover, AMPK can also activate autophagy by direct phosphorylation of ULK1 (a mammalian homologue of yeast Atg1) and Vps34 complexes.^{16, 17} It will be interesting to determine the phosphorylation status of ULK1 and Vps34 complexes in LPS and LPS/D-GalN-treated mouse liver in the future.

The finding that an increase in autophagy when Beclin 1 was overexpressed using an adeno-associated virus (rAAV) inhibited LPS/D-GalN-induced liver injury is quite significant.⁸ These findings may lead to highly translational therapeutic approaches to treat acute cytokine-mediated tissue injury by inducing autophagy. We have previously demonstrated that induction of autophagy by rapamycin significantly attenuates acetaminophen and alcohol-induced liver injury.^{9, 10} It remains to be tested whether rapamycin would also be beneficial for cytokine-mediated acute liver injury. More recently, a small peptide autophagy inducer, Tat-beclin 1, which was derived from a region of the autophagy protein Beclin 1, was shown to have great potential to treat polyglutamine expansion protein aggregates and the replication of several pathogens in vitro.¹⁸ With more novel agents

being developed for inducing autophagy, these compounds will open a new therapeutic avenue for treating liver diseases.

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Figure 1. The intracellular death signaling regulates cross talk of apoptosis and autophagy in liver injury

TNF- α binds to its receptor to stimulate receptor trimerization and the assembly of the death-inducing signaling complex. In this complex, FADD recruits caspase-8 (Casp-8) to promote Casp-8 oligomerization and self-activation, which is inhibited by c-FLIP. p62 binds to polyubiquitinated Casp-8 and recruits Casp-8 to the autophagosomal membranes through its direct interaction with LC3-II to promote Casp-8 activation. Activated Casp-8 cleaves Bid to tBid to trigger the mitochondrial release of cytochrome c and SMAC resulting in Casp-9 and its downstream Casp-3/6/7 activation and apoptosis. Deleting Atg7 in the mouse liver leads to the overactivation of JNK in LPS/D-GalN-treated mice, which promotes c-FLIP degradation and promotes Casp-8 activation. In contrast, overexpression of an autophagy protein Beclin-1 inhibits LPS/D-GalN-induced Casp-8 activation and liver injury.