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A Gene Transcription Program Decides the Differential Regulation of Autophagy by Acute vs Chronic Ethanol?

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Acute and chronic alcohol abuse lead to liver injury associated with steatosis, alcoholic hepatitis, liver fibrosis, cirrhosis, and cancer (Gao and Bataller, 2011, Williams et al., 2014). The mechanisms for alcohol-induced liver injury have been extensively studied and significant progress has been made. These mechanisms include oxidative and non-oxidative metabolism of ethanol, increased oxidative stress and oxidative damage, organelle damage (mitochondria and lysosomes), endoplasmic reticulum (ER) stress, cytokine release, inflammation, and subsequent apoptotic and necrotic cell death (Molina et al., 2002, Williams et al., 2014, Gao and Bataller, 2011). Intriguingly, only a small portion of alcohol drinkers develop alcoholic liver disease such as alcoholic hepatitis and fibrosis, suggesting other protective mechanisms exist (Gao and Bataller, 2011, Williams et al., 2014). Cells can adapt to these detrimental effects induced by alcohol consumption by activating cellular protective mechanisms. One of these important cellular adaptive mechanisms is autophagy, which is a genetically programmed, highly conserved intracellular lysosomal degradation pathway (Xie and Klionsky, 2007). We previously demonstrated that autophagy is activated in mouse livers and primary cultured mouse and human hepatocytes in response to acute alcohol (Ding et al., 2010, Ni et al., 2013a). Autophagy helps to remove ethanol-induced damaged mitochondria and excess lipid droplets via selective mitophagy and lipophagy (Ni et al., 2013a, Ding et al., 2010, Ding et al., 2011). While two independent groups reported that chronic ethanol exposure enhanced autophagy flux in mice (4 week alcohol feeding) and increased autophagosome numbers in rats (10 week alcohol feeding) (Lin et al., 2013, Eid et al., 2013), earlier studies indicated that chronic ethanol exposure disrupts lysosome function and biogenesis (Kharbanda et al., 1995). Moreover, chronic ethanol exposed animals and heavy drinkers develop hepatomegaly with increased hepatic protein accumulation (Baraona et al., 1975). All of these observations suggest a defect in autophagy in chronic alcohol exposure conditions. Therefore, the autophagy status during chronic alcohol consumption is still controversial and remains a mystery.

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In this issue of *Alcoholism: Clinical and Experimental Research*, Thomes *et al.* compared the autophagy status and corresponding underlying mechanisms in acute and chronic ethanol fed mice (Paul G. Thomes and Donohue, 2015). They confirmed that acute ethanol administration to mice (single gavage, 6g/kg body weight) increased autophagy flux in the livers based on increased autophagosome numbers, autophagosome and lysosome fusion, and increased GFP-LC3 protein turnover. Interestingly, they found that mice administered with ethanol for 35–62 days had decreased lysosome numbers and autophagosome-lysosome co-localization as well as decreased GFP-LC3 turnover in the livers. However, these mouse livers had increased hepatic p62/SQSTM1 and LC3-II proteins. These data suggest that chronic ethanol exposure may impair autophagy flux at the late autophagy phase such as at the lysosome. They further found that chronic ethanol exposure decreased 20S proteasome activity but did not affect the mRNA levels of LC3 or p62/SQSTM1. This indicates that LC3-II accumulation was due to impaired lysosomal degradation of LC3-II rather than upregulated autophagosome biogenesis after chronic ethanol exposure. Taken together, these results suggest that acute and chronic alcohol may differentially regulate autophagy in the liver. However, it should be noted that autophagy is a highly dynamic process, and liver autophagy is even more complicated because it is affected by nutrient availability, circadian rhythm and cytochrome P450-mediated metabolites. Moreover, the liver has a unique zonation pattern characterized by portal, midzone and centra-lobular areas, which have different oxygen, fatty acid, cytochrome P450 enzyme and glutamine levels and may result in different levels of autophagy (Ni *et al.*, 2013b). Moreover, most current autophagy assays use either fluorescence microscopy to determine GFP-LC3 labeled autophagosomes or western blot analysis to quantify the protein levels of LC3-II and p62/SQSTM1. These assays only look at the steady state autophagy status using one time point. However, the nature of autophagy is dynamic, making it difficult to monitor *in vivo* in livers after alcohol exposure. Therefore, more quantitative autophagy assays capable of reflecting the dynamic nature of autophagy are needed to further determine autophagy status in acute and chronic alcohol exposures in the liver.

The lysosome contains more than 50 acid hydrolases and is the terminal component of autophagy. Increasing evidence suggests that transcriptional regulation of genes for lysosome biogenesis and autophagy plays a critical role in autophagy (Settembre *et al.*, 2013). The transcription factor EB (TFEB) is a basic helix-loop-helix leucine zipper transcription factor and belongs to the coordinated lysosomal expression and regulation (CLEAR) gene network, which is considered to be a master regulator for transcription of genes for lysosome biogenesis and autophagy (Settembre *et al.*, 2012, Settembre *et al.*, 2011). TFEB binds specifically to a 10-bp (GTCACGTGAC) motif found in the promoter regions of many genes encoding for lysosomal biogenesis and autophagy induction (Settembre *et al.*, 2011). In response to increased autophagic degradation needs, TFEB coordinates an efficient transcriptional program to upregulate genes that are responsible for both early (autophagosome formation) and late (lysosome biogenesis) phases of autophagy. Nuclear localization and activity of TFEB are regulated by serine phosphorylation mediated by at least three kinases: the extracellular signal-regulated kinase 2 (ERK2), mechanistic target of rapamycin complex 1 (mTORC1) and protein kinase C β (PKC β) (Settembre *et al.*, 2013). TFEB is phosphorylated at Ser142 by ERK2 and at both Ser142 and Ser211 by

mTORC1, which promotes TFEB binding with the cytosolic chaperone 14-3-3 and sequesters TFEB in the cytosol, resulting in its inactivation (Settembre et al., 2011, Settembre et al., 2013). Under nutrient rich conditions, TFEB interacts with active Rag heterodimers resulting in the recruitment of TFEB to lysosomes where mTORC1 phosphorylates and inhibits TFEB (Martina and Puertollano, 2013). Recently, calcium release from lysosomes through mucolipin 1 (MCOLN1) was reported to dephosphorylate TFEB via activation of the phosphatase calcineurin, which subsequently activates TFEB and induces autophagy (Medina et al., 2015). Moreover, during Pink1-Parkin-mediated selective mitophagy, TFEB is dephosphorylated and activated in a Parkin-, PINK1-, Atg5- and Atg9A-dependent manner (Nezich et al., 2015).

It would be interesting to see if differential effects on autophagy during acute and chronic alcohol exposure are mediated by TFEB. Thomes et al. determined TFEB protein levels in cytoplasmic and nuclear fractions from acute and chronic alcohol-treated mouse livers, and they found that nuclear protein levels of TFEB were increased in acute alcohol treated mouse livers (Paul G. Thomes and Donohue, 2015). In contrast, chronic alcohol consumption decreased nuclear TFEB protein levels in mouse livers. Consistent with the negative regulation of TFEB by ERK2, they further found that the phosphorylation levels of ERK1/2 decreased in acute alcohol-treated, but increased in chronic alcohol-treated, mouse livers. Based on the above data, they proposed that a burst of oxidative stress may lead to elevated TFEB nuclear translocation and contribute to autophagy activation through the inactivation of ERK following acute alcohol consumption. Under chronic alcohol consumption conditions, the sustained elevation of oxidative stress together with other factors, e.g. inhibited lysosomal function due to steatosis, mTOR activation by increased intrahepatic leucine, and disrupted hepatic protein trafficking by ethanol metabolites, may contribute to decreased TFEB translocation resulting in autophagy inhibition.

While this study made several interesting important observations for differential cellular localization of TFEB and autophagy markers in acute vs. chronic alcohol exposure conditions, several important questions remain unanswered. Like many other transcription factors, while nuclear translocation of TFEB is necessary for its activation, its transcriptional activity is also dependent on many co-activators or repressors. Unfortunately, no functional studies for TFEB, such as determination of expression of lysosomal and autophagy-related genes, were conducted in this study. As discussed above, in addition to ERK, TFEB can also be regulated by mTORC1 and the activity of mTORC1 was not determined in this study. Interestingly, the necroptosis protein receptor-interacting kinase 1 (RIPK1) has been recently reported to negatively regulate TFEB via activating ERK, resulting in repression of autophagy induction in some cultured cancer cells (Yonekawa et al., 2015). Roychowdhury et al. showed that chronic ethanol-induced liver injury is dependent on RIPK3 but not RIPK1 (Roychowdhury et al., 2013). Therefore, it would be interesting to investigate the role of RIPK1 in the regulation of TFEB after both acute and chronic alcohol exposure in the future. As discussed above, calcium-mediated calcineurin activation promotes TFEB activation. Disruption of intracellular calcium levels in liver after alcohol exposure has also been reported (Baffy et al., 1990, Zheng et al., 2006, Yan et al., 2007). Therefore, it would also be intriguing to determine whether altered intracellular calcium signaling is involved in TFEB regulation after both acute and chronic ethanol exposure. Moreover, we recently

demonstrated that chronic alcohol feeding plus acute binge (Gao-binge alcohol model) induced Parkin mitochondrial translocation and mitophagy (Williams et al., 2015). The Gao-binge alcohol model is designed to mimic human chronic alcohol plus binge conditions, and it would also be interesting to determine the status of TFEB and autophagy as well as the role of Parkin in regulating TFEB activation in this model in the future.

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Abbreviations

TFEB	transcription factor EB
ERK	extracellular signal-regulated kinase
mTORC1	mechanistic target of rapamycin complex 1
RIPK1	protein receptor-interacting kinase 1

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