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Invited perspective

Targeting Pink1-Parkin-mediated mitophagy for treating liver injury

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ABSTRACT

Alcoholic liver disease and acetaminophen overdose are common causes of severe liver disease and liver failure in the United States for which there is no cure. Therefore, development of new therapeutic strategies for treatment of liver injury caused by acetaminophen and alcohol is needed. We demonstrated that autophagy protects against alcohol and acetaminophen-induced liver injuries by removing damaged mitochondria via mitophagy, which is a selective form of autophagy specific for degradation of damaged mitochondria. Parkin is well-known to be required for mitophagy induction in *in vitro* models, and we previously showed that the Parkin-mediated mitophagy pathway likely plays a protective role against alcohol and acetaminophen-induced liver injuries. Therefore, pharmacological upregulation of the Parkin-mediated mitophagy pathway in the liver may provide a novel and effective therapeutic option for treatment of acetaminophen and alcohol-induced liver injuries. In this review, we discuss regulation of Parkin-mediated mitophagy and possible therapeutic targets of intervention in this pathway.

1. Introduction

Autophagy is a protective process that provides nutrients during starvation by degrading and recycling excess and/or damaged organelles [1]. Autophagy can be both selective and non-selective [1,2]. We demonstrated that autophagy protects against alcohol and acetaminophen (APAP)-induced liver injuries by removing damaged mitochondria by mitophagy, a selective form of autophagy specific for degradation of damaged mitochondria [3–5]. However, the mechanism for how these mitochondria were removed by mitophagy was unknown.

Parkin was shown to be required for induction of mitophagy in *in vitro* models [6]. Parkin is an E3 ubiquitin ligase encoded by the *Park2* gene and is a 465 amino acid protein [7]; it is well known to be protective in the brain because loss of Parkin leads to development of Autosomal Recessive Parkinson's disease [8].

Even though the majority of research regarding Parkin has been related to neurodegenerative diseases such as Parkinson's disease, we found that Parkin is also highly expressed in the liver in mice [9]. Therefore, we investigated the role of Parkin in mitophagy induction as a protective mechanism against alcohol and APAP-induced liver injuries, and we found that Parkin-mediated mitophagy likely protects against alcohol and APAP-induced liver injuries by removing damaged mitochondria [10,11].

APAP overdose-induced acute liver injury/failure and alcoholic liver disease (ALD) are two major liver diseases in the United States and European countries. ALD is a chronic liver disease that is characterized by hepatic steatosis, hepatocyte cell death, inflammation, fibrosis and eventual liver cirrhosis and hepatocellular carcinoma. APAP-induced liver injury is characterized by massive central lobular hepatocyte necrosis. Currently, no successful treatments for APAP-induced liver damage and ALD are available, so novel therapeutic interventions are needed. Mitochondrial damage is well known to play a major role in both alcohol and APAP-mediated liver injuries [12,13]. Therefore, upregulation of the Pink1-Parkin-mediated mitophagy pathway may be a beneficial therapeutic target to ameliorate progression of APAP or alcohol-mediated liver injury by removing damaged mitochondria. There are several layers of regulation for the Parkin-mediated mitophagy pathway that could act as potential therapeutic targets, which are the foci of this review.

Abbreviations: APAP, acetaminophen; ALD, alcoholic liver disease; BAG4, Bcl2-associated athanogene 4; CCCP, carbonyl-cyanide *m*-chlorophenyl hydrazine; Clec16a, c-type lectin domain family 16, member A; Dfcp1, double FYVE-containing protein 1; DUB, deubiquitinating enzyme; IBR, in-between RING; JNK, c-JUN N-terminal kinase; KO, knockout; Mcl-1, myeloid cell leukemia 1; MFN1\2, mitofusin 1 and 2; NBR1, neighbor of BRCA Gene 1; Nrdp1, neuregulin receptor degradation protein 1; PINK1, phosphatase and tensin homolog (PTEN)-induced kinase 1; PARL, presenilin-associated rhomboid-like protein; RING, really interesting new gene; siRNA, small-interfering RNA; Tax1BP1, tax1-binding protein 1; TBK1, tank-binding kinase 1; TOM20, translocase of outer mitochondrial membrane 20; TOM, transporter outer membrane; TIM, transporter inner membrane; UPS, ubiquitin proteasome system; UBL, ubiquitin-like; ULK1, unc-51 like autophagy activating kinase 1; VDAC, voltage-dependent anion channel; WIPI1, WD repeat domain phosphoinositide-interacting protein 1; WT, wild-type.

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2. The role of Parkin and mitophagy in acetaminophen and alcohol-induced liver injuries

We investigated the role of Parkin and mitophagy in alcohol and APAP-induced liver injuries using wild-type (WT) and Parkin knockout (KO) mice. Parkin protects against alcohol-induced liver injury and steatosis via mitophagy and maintenance of mitochondrial function [10]. Parkin KO mice have more liver injury and steatosis after alcohol treatment compared to WT mice because they have more severely damaged mitochondria and decreased mitochondrial function after treatment with alcohol, which is likely due to decreased mitophagy in addition to reduction in other functions of Parkin for maintaining mitochondrial integrity [10]. Therefore, pharmacological upregulation of Parkin-induced mitophagy may be a novel and effective therapeutic target for reducing alcohol-mediated liver injury and steatosis.

Using a mouse APAP-induced liver injury model, we demonstrated that APAP treatment increases Parkin mitochondrial translocation and levels of ubiquitinated mitochondrial proteins, suggesting that Parkin-mediated mitophagy may also occur in hepatocytes after APAP exposure [11]. Using an adenovirus shRNA against Parkin to knockdown Parkin in mouse livers, we found that these mice have decreased mitophagy and are more susceptible to APAP-induced liver injury. These results suggest that Parkin-mediated mitophagy protects against APAP-induced liver injury. However, APAP-treated-Parkin KO mice revealed surprising and intriguing results. Unlike liver shRNA Parkin knockdown mice, we found that Parkin KO mice do not have exacerbated APAP-induced liver injury but instead are protected against APAP-induced liver injury compared to WT mice. Subsequent studies revealed that Parkin KO mice have decreased c-JUN N-terminal kinase (JNK) activation along with increased Myeloid cell leukemia 1 (Mcl-1) expression and hepatocyte proliferation compared to WT mice, which all contribute to their protection against APAP-induced liver injury [11]. These opposing responses to APAP overdose in Parkin KO and acute knockdown mice are likely due to the compensatory adaptive responses in Parkin KO mice. Mice with acute Parkin knockdown may lack time needed to develop adaptive responses that are present in Parkin KO mice. Indeed, these compensatory protective mechanisms present in Parkin KO mice after APAP treatment are absent in mice with acute Parkin knockdown. Thus, the decreased mitophagy in acute Parkin knockdown mice likely had a role in their increased liver injury after APAP treatment due to the lack of removal of APAP-induced damaged mitochondria compared to WT mice. Therefore, pharmacological upregulation of Parkin-induced mitophagy may help reduce APAP-induced liver injury by removing damaged mitochondria.

Overall, our animal studies suggest that targeting the Parkin-induced mitophagy pathway may be a novel therapeutic option for prevention and / or treatment of severe liver disease caused by APAP or alcohol.

3. Regulation of Parkin-induced mitophagy and potential therapeutic targets for treatment of APAP and alcohol-induced liver injuries

Parkin is a cytosolic E3 ubiquitin ligase that translocates to depolarized mitochondria to initiate mitochondrial degradation via mitophagy [6]. Parkin-dependent mitophagy is regulated by many factors, with one of the most important being phosphatase and tensin homolog (PTEN)-induced kinase 1 (PINK1), which acts upstream of Parkin in the mitophagy pathway. Parkin directly interacts with PINK1 [14], and overexpression of PINK1 alone can initiate translocation of Parkin to mitochondria without mitochondrial damage [15]. The specificity for PINK1's role in recruiting Parkin is further shown by ectopic expression of PINK1 on peroxisomes, which results in recruitment of Parkin and degradation of PINK1-expressing peroxisomes by autophagy [16]. PINK1 promotes Parkin-mediated mitophagy by recruiting Parkin to damaged mitochondria.

PINK1 is normally cleaved and degraded in healthy mitochondria. However, PINK1 becomes stabilized on the outer mitochondrial membrane when mitochondria are depolarized, which signals Parkin recruitment to damaged mitochondria. To induce PINK1 cleavage in healthy mitochondria, the transporter outer membrane (TOM) complex on the outer mitochondrial membrane imports PINK1 into the inner mitochondrial membrane through the transporter inner membrane (TIM) complex where it is degraded by PARL (presenilin-associated rhomboid-like protein) [17]. Cleavage by PARL removes the N-terminus of PINK1, and the truncated PINK1 protein is released into the cytosol where it is degraded by the ubiquitin proteasome system (UPS) through the N-end rule mechanism [18]. However, when mitochondria are depolarized, import of PINK1 into the inner mitochondrial membrane is blocked, so PINK1 is no longer cleaved and degraded and instead becomes stabilized on the outer mitochondrial membrane [17]. Accumulation of misfolded proteins in the mitochondria can also stabilize PINK1 on the outer mitochondrial membrane without mitochondrial depolarization. However, the mechanism for PINK1 stabilization in this model is unknown [19]. In addition to its stabilization on the outer mitochondrial membrane, PINK1 must also phosphorylate itself on Ser228 and Ser402 to recruit Parkin to mitochondria [20]. Auto-phosphorylation of PINK1's Ser228 and Ser402 residues increases PINK1-induced recruitment and phosphorylation of Parkin and ubiquitin, which is necessary for recruitment of Parkin to damaged mitochondria for induction of mitophagy [20,21].

In addition to mediating recruitment of Parkin to mitochondria, PINK1 also activates Parkin's E3 ligase activity to induce ubiquitination of mitochondrial outer membrane proteins and subsequent degradation of mitochondria in autophagosomes. PINK1-mediated phosphorylation of Parkin at Ser65 in its ubiquitin-like (UBL) domain is shown to activate Parkin's E3 ligase activity [22–24]. In addition to phosphorylating Parkin, PINK1 also phosphorylates ubiquitin at Ser65 to allow for greater recruitment and activation of Parkin. Ser65-phosphorylated ubiquitin binds to Ser65-phosphorylated Parkin with 21-fold greater affinity compared to non-phosphorylated Parkin, resulting in greater levels of Parkin activity and mitochondrial protein ubiquitination [25,26]. PINK1-induced phosphorylation of both Parkin and ubiquitin at Ser65 acts as a feed-forward loop for ubiquitination of substrate proteins on the outer mitochondrial membrane. Once Parkin is recruited to the mitochondria and activated by Ser65 phosphorylation, PINK1 phosphorylates ubiquitin (Ser65) attached to mitochondrial proteins, which tethers Parkin to the mitochondria allowing for further Parkin recruitment and ubiquitination of outer mitochondrial membrane proteins by Parkin to initiate mitophagy [27,28]. Due to its requirement for Parkin recruitment to damaged mitochondria and activation of its E3 ligase activity, PINK1 is an optimal therapeutic target for upregulating the mitophagy pathway to help reduce alcohol and APAP-induced liver injuries. Upregulation of mitophagy was recently accomplished *in vitro* by stabilizing PINK1 on the outer mitochondrial membrane via an ATP analog, kinetin triphosphate, which recruits Parkin to the mitochondria and prevents cell death in carbonyl-cyanide m-chlorophenyl hydrazine (CCCP)-treated Parkin-expressing HeLa cells [29]. Future work is needed to further validate the protective role of kinetin triphosphate to induce PINK1-Parkin mediated mitophagy *in vivo* in APAP or alcohol models. In addition to PINK1 stabilization, activation of PINK1 kinase activity could also be a therapeutic target since PINK1 auto-phosphorylation and phospho-