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Research Paper

# Mitochondrial dysfunction and tissue injury by alcohol, high fat, nonalcoholic substances and pathological conditions through post-translational protein modifications

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ABSTRACT

Mitochondria are critically important in providing cellular energy ATP as well as their involvement in anti-oxidant defense, fat oxidation, intermediary metabolism and cell death processes. It is well-established that mitochondrial functions are suppressed when living cells or organisms are exposed to potentially toxic agents including alcohol, high fat diets, smoking and certain drugs or in many pathophysiological states through increased levels of oxidative/nitrative stress. Under elevated nitroxidative stress, cellular macromolecules proteins, DNA, and lipids can undergo different oxidative modifications, leading to disruption of their normal, sometimes critical, physiological functions. Recent reports also indicated that many mitochondrial proteins are modified via various post-translation modifications (PTMs) and primarily inactivated. Because of the recently-emerging information, in this review, we specifically focus on the mechanisms and roles of five major PTMs (namely oxidation, nitration, phosphorylation, acetylation, and adduct formation with lipid-peroxides, reactive metabolites, or advanced glycation end products) in experimental models of alcoholic and nonalcoholic fatty liver disease as well as acute hepatic injury caused by toxic compounds. We also highlight the role of the ethanol-inducible cytochrome P450-2E1 (CYP2E1) in some of these PTM changes. Finally, we discuss translational research opportunities with natural and/or synthetic anti-oxidants, which can prevent or delay the onset of mitochondrial dysfunction, fat accumulation and tissue injury.



*Abbreviations:* ; AA-AGE, acetaldehyde-derived advanced glycation end product; ACR, acrolein; AFLD, alcoholic fatty liver disease; AGE-albumin, advanced glycation end product-albumin adduct; ALDH2, mitochondrial low-Km aldehyde dehydrogenase 2; AMPK, AMP-activated protein kinase; APAP, acetaminophen; Complex I, NADH-dependent ubiquinone oxidoreductase; Complex III, ubiquinone cytochrome bc1 oxidoreductase; Complex IV, cytochrome c oxidase; Complex V, ATP synthase; CYP2E1, ethanol-inducible cytochrome P450 2E1 isozyme; DILI, drug-induced liver injury; DTT, dithiothreitol; eNOS, endothelial NOS; ER, endoplasmic reticulum; ERK, extracellular signal regulated protein kinase; ETC, electron transport chain; Gpx, glutathione peroxidase; GSH, glutathione; 4-HNE, 4-hydroxynonenal; HIF, hypoxia-inducible factor; ICDH, isocitrate dehydrogenase; I-kB, inhibitor protein of NF-κB; iNOS, inducible nitric oxide synthase; I/R, ischemia–reperfusion; JNK, c-Jun N-terminal protein kinase; Keap1, Kelch-like ECH-associated protein 1; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MDMA, 3,4-methylenedioxymethamphetamine; MGO, methylglyoxal; mito-CP, mitochondria-targeted carboxy-proxyl; mitoQ, mitochondria-targeted ubiquinone; MPT, mitochondrial permeability transition; mtGSH, mitochondrial glutathione; NAC, *N*-acetylcysteine; NAFLD, nonalcoholic fatty liver disease; NF-κB, nuclear factor-κB; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NEL-adduct, *N*-ethyllysine adduct; nNOS, neuronal NOS; NO, nitric oxide; Nrf2, nuclear factor (erythroid-derived 2)-like 2; 4-ONE, 4-oxonon-2-enal; p38K, p38 protein kinase; PGC1α, peroxisomal proliferator activated receptor gamma coactivator-1α; PKC, protein kinase C; Prx, peroxiredoxin; PTEN, lipid phosphatase and tensin homolog; RAGE, receptor for advanced glycation end product; RNS, reactive nitrogen species; ROS, reactive oxygen species; *S*-NO-Cys, *S*-nitrosylated Cys; SAMe, *S*-adenosylmethionine; SOD, superoxide dismutase; SREBP, sterol regulated element binding protein; Thiolase, 3-ketoacyl-CoA thiolase; TIMP, tissue inhibitor of metalloproteinase 3; $TPP^+$, triphenylphosphonium cation.

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## Introduction

All living organisms require exogenous foods/nutrients for producing energy in the form of ATP, which is needed for numerous cellular functions. Most of the cellular energy is efficiently produced in specialized organelles mitochondria by oxidative phosphorylation. In addition to energy production, mitochondria play an important role in other cellular functions such as fatty acid oxidation, anti-oxidant defense, intermediary metabolism including ammonia and glutamate detoxification, synthesis of heme and steroids, cell death process, and autophagy [1]. Recent reports showed that mitochondria also undergo constant morphological changes with fusion and fission, following the exposure to toxic agents and/or under pathological conditions [2,3]. After decreased glucose supply like during fasting or inefficient oxidative phosphorylation in certain disease states, the mitochondrial fat oxidation pathway becomes important in providing an alternative source of energy (e.g., ketone bodies) [1]. Without these energy supply mechanisms due to suppressed mitochondrial function (i.e., mitochondrial dysfunction), living cells would die or become susceptible to cell death processes (necrosis and apoptosis). In fact, it is well-established that mitochondrial function in different tissues is significantly suppressed in numerous medical disorders such as metabolic syndrome including obesity/diabetes, cardiovascular disorders, ischemia reperfusion injury (I/R)[1] and cancer as well as many neurological disorders like Alzheimer's disease and Parkinson's disease [4–12], although etiological causes of each disease are different.

Hepatic mitochondrial abnormalities are observed in both alcoholic fatty liver disease (AFLD) [4–6], nonalcoholic fatty liver disease (NAFLD) [7–9] and acute liver injury. As a result, it is conceivable to observe decreased ATP levels and increased fat accumulation (micro-vesicular and macro-vesicular steatosis) in the liver under these conditions. Continued mitochondrial dysfunction with increased oxidative stress also sensitizes the hepatocytes to subsequent necrosis and/or apoptosis of hepatocytes, which likely lead to activation of resident liver macrophage (i.e., Kupffer cells) and recruitment of infiltrating immune cells into the liver with elevated levels of hepatic inflammation (steatohepatitis) and pro-inflammatory cytokines/chemokines [13–15]. Consequently, hepatic stellate cells can be activated and transformed into myofibroblast-like cells, producing pro-fibrotic cytokines such as transforming growth factor-beta and platelet derived growth factor, leading to hepatic fibrosis/cirrhosis and cancer. These sequential events can be observed in both AFLD [13–15] and NAFLD [16,17]. In addition, acute or sub-chronic exposure to various hepatotoxic compounds, including clinically-used drugs, such as acetaminophen (APAP) [18–20], a major ingredient of Tylenol®, an anti-breast cancer agent tamoxifen [21], an anti-retroviral drug zidovudine (AZT) [22] and antidepressants [23], can cause mitochondrial dysfunction, contributing to liver injury with or without fat accumulation, depending on the injurious agent, as extensively reviewed [24–26]. These hepatotoxic agents and pathological conditions are known to elevate the levels of reactive oxygen and nitrogen species (ROS/RNS) and nitroxidative stress through the suppression of the mitochondrial electron transport chain (ETC) and induction/activation of NADPH oxidase, cytochrome P450 isozymes including ethanol-inducible P450-2E1 (CYP2E1) and CYP4A, xanthine oxidase, and inducible nitric oxide synthase (iNOS). Although increased nitroxidative stress can oxidatively damage mitochondrial DNA and lipids, the majority of insults can also take place at the protein levels through different forms of post-translational modification (PTM) [26,27]. Because of the newly-emerging information on redox-related protein modifications, we briefly describe five major forms of PTM and functional consequences of some modified mitochondrial proteins in the experimental models of the AFLD, NAFLD and acute liver injury (Fig. 1). In addition, we highlight potential roles of CYP2E1 in promoting various PTMs.

## Post-translational modifications of mitochondrial proteins

### *Oxidation of mitochondrial proteins*

Under normal conditions, transiently elevated ROS is known to be involved in cellular signaling pathways [28,29] and mitochondrial functions are correctly maintained through proper redox balance. However, chronic and/or binge alcohol, high fat diets, tobacco smoking, or certain hepatotoxic drugs can directly damage the mitochondrial ETC, producing greater amounts of ROS leaked from the ETS [24–27]. Without proper counter-balance by various cellular anti-oxidants, the persistent imbalance in cellular redox states result in decreased levels of mitochondrial antioxidants including mitochondrial glutathione (mtGSH), which serves as a critical determinant between toxic damage and cellular protection [30]. When the cellular defense system is overwhelmed, greater amounts of ROS and RNS remain elevated, ultimately leading to increased nitroxidative stress.

It is well-established that many amino acids such as cysteine (Cys), methionine (Met), histidine (His), proline (Pro), lysine (Lys), tyrosine (Tyr), phenylalanine (Phe), threonine (Thr) and tryptophan (Trp) in most proteins can also be redox regulated. As recently reviewed [26,27], Cys residue(s) can be oxidatively modified in many forms [sulfenic acid, disulfide, sulfinic/sulfonic acids, NO- or peroxynitrite-dependent *S*-nitrosylation, NO-independent ADP-ribosylation, mixed disulfide formation between Cys residues and glutathione (glutathionylation), cysteine (cystathionylation), succinic acid, myristic acid [31] or palmitic acid (Cys-palmitoylation) [32] prior to membrane attachment or cellular trafficking].

Since the sensitive methods to detect redox-regulated Cys residues in cellular proteins were extensively described in our previous reviews [26,33], we will only highlight the functional consequences of oxidative modification of Cys residues in some proteins. If one of these amino acids serves as the active site or is located near the active site of certain enzymes, it is highly likely that oxidative modifications of these amino acids can result in their inactivation, as shown by the oxidation and/or *S*-nitrosylation of Cys residues including the active site Cys in the mitochondrial aldehyde dehydrogenase (ALDH2) [34] and 3-ketoacyl CoA-thiolase (thiolase) [34] in binge alcohol-exposed rodents. In fact, mass-spectral analysis revealed that more than 87 mitochondrial and 60 cytosolic proteins were oxidatively-modified in alcohol-exposed rodents [34,35]. The rates of protein oxidation in CYP2E1-containing E47-HepG2 hepatoma cells [36] exhibited a dose- and time- dependent pattern in response to alcohol as well as the presence of CYP2E1 [37]. Similar numbers of oxidatively-modified mitochondrial proteins were also identified by mass-spectral analysis in mice with I/R injury [38] and rats exposed to 3,4-methylenedioxymethamphetamine (MDMA) [39]. The number of oxidatively-modified mitochondrial proteins we characterized may represent a small fraction of the estimated number of 1100–1400 total mitochondrial proteins [40,41]. However, we believe that the actual number of oxidized proteins could be significantly higher than the proteins originally reported [34,38,39], because of the technical limitations in the identification and purification of oxidized proteins and detection methods, especially for those proteins expressed in low abundance, as previously discussed [26,33].

In the case of oxidative inactivation of mitochondrial ALDH2 through active site Cys modification [42], immunoblot analysis of the immunoaffinity purified ALDH2 proteins by using the specific