Current Research Trends in the Exploration of Therapeutic Targets for Liver Disease

Sestrin2: A Promising Therapeutic Target for Liver Diseases

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Sestrin2 (Sesn2), a highly conserved antioxidant protein, is induced by various stresses, including oxidative and energetic stress, and protects cells against those stresses. In normal physiological conditions, redox-homeostasis plays an essential role in cell survival and performs the cellular functions to protect the cells against oxidative damage. The liver is susceptible to oxidative stress, since it is responsible for xenobiotic detoxification and energy metabolism. For this reason, oxidative stress is associated with the pathogenesis of liver diseases. Recently, the role of Sesn2 has been investigated in liver injury and related diseases. In this paper, we review the role of Sesn2 in the pathophysiology of liver diseases and the potential clinical applications of Sesn2 as a therapeutic target to prevent/treat liver diseases. This article promotes our understanding of liver disease progression and advances the development of strategies for pharmacological intervention.

Key words: sestrin; hydrogen peroxide; peroxiredoxin; oxidative stress; liver disease

1. INTRODUCTION

Sestrin2 (Sesn2) has recently been identified as an evolutionarily conserved antioxidant protein whose expression is upregulated in cells exposed to various stresses, such as oxidative and energetic stress.1) It diminishes reactive oxygen species (ROS) accumulation, protects cells against oxidative damage, and regulates cell proliferation and viability. It is well documented that oxidative stress plays an important role in the pathogenesis of many organ diseases. The liver is a metabolically active organ; hence, it is more susceptible to oxidative damage that can result in liver diseases, including hepatitis, fibrosis, cirrhosis, and even cancer. Since Sesn2 is regarded as a key modulator of oxidative stress, it is essential to investigate the roles of Sesn2 in liver diseases. Growing evidence shows that Sesn2 is associated with many liver diseases. In this review, we discuss the role of Sesn2 in liver pathophysiology and the potential application of Sesn2 as a therapeutic target to prevent and treat liver diseases.

2. THE FUNCTION OF SESTRINS IN HYDROGEN PEROXIDE AND REDOX-HOMEOSTASIS SIGNALING

Among ROS, hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is recognized as an important signaling molecule.2–4) H\textsubscript{2}O\textsubscript{2} has defined targets such as protein thiol moieties that produce sulfur oxidation products, including disulfides, sulfenic acid (–SOH), sulfonic acid (–SO\textsubscript{3}H), or sulfoxonic acid (–SO\textsubscript{3}H\textsubscript{2}).5) The removal of H\textsubscript{2}O\textsubscript{2} from cell occurs through multiple routes, including breakdown by antioxidant enzymes such as glutathione peroxidase or catalase or reaction with non-enzymatic antioxidants such as vitamins and glutathione (GSH).6) Moreover, a family of mammalian peroxiredoxin (Prx I to Prx VI) scavenge H\textsubscript{2}O\textsubscript{2} and alkyl hydroperoxides.7–9)

Prxs, as small thiol-containing peroxidases, contain conserved cysteine residues in the NH\textsubscript{2}-terminal region, which is a primary target of H\textsubscript{2}O\textsubscript{2}.10) In the catalytic cycle of Prxs, the conserved NH\textsubscript{2}-terminal cysteine is first converted to cysteine sulfenic acid (Cys-SOH), which then reacts with the conserved COOH-terminal cysteine of the other subunit to produce an intermolecular disulfide bond. This disulfide bond can be reduced by thioredoxin (Trx) coupled with Trx reductase and reduced nicotinamide adenine dinucleotide phosphate (NADPH).11) In the presence of high levels of H\textsubscript{2}O\textsubscript{2}, Prx loses peroxidase activity owing to the overoxidation of cysteine to sulfenic acid (Cys-SO\textsubscript{3}H) or sulfonic acid (Cys-SO\textsubscript{3}H\textsubscript{2}). Trx cannot reduce sulfenic acid of overoxidized Prxs, and such overoxidation has been considered a consequence of the permanent inactivation of Prxs.12) However, it was reported that inactivation of these enzymes by overoxidation of the active thiol can be reversed by sulfiredoxin (Srx) in an ATP- and Mg\textsuperscript{2+}-dependent manner12) (Fig. 1).

It was recently reported that Sesns, evolutionarily conserved antioxidant proteins, also reduce cysteine sulfenic acid levels and inhibit ROS production by regenerating overoxidized Prxs, despite the fact that Sesns share no structural homology with Srx.13) Sesns act as antioxidant proteins that protect cells against hypoxia, genotoxic, and oxidative stress.14)

3. THE ISOFORMS OF SESTRINS

Most vertebrates express three isoforms of Sesns,14–16) while most non-mammalian invertebrate species contain only a single Sesn gene.17) Sesn1 also known as PA26 has been identified as a member of the growth arrest and DNA-damage inducible genes (GADD) family, whose expression is mainly
regulated by p53. In addition, Sesn2 has been shown to have cytoprotective activity against hydrogen peroxide or ischemia, as well as genotoxic damage. Sesn3 is considered a novel PA26-related gene, through analysis of the PA26 gene structure and is regulated by forkhead box O (FOXO) transcription factors.  

4. SESTRIN2 IN LIVER PATHOPHYSIOLOGY  

4.1. Hepatoprotective Effect of Sestrin2  
NF-E2-related factor-2 (Nrf2), a member of the cap ‘n’ collar family of bZIP transcription factors, is activated in response to oxidative stress in various cells and tissues. Hence, Nrf2 activation has been extensively investigated to prevent and treat liver diseases. Previously, we reported that Nrf2 pathway regulates Sesn2 gene induction. Moreover, Sesn2 small interfering RNA (siRNA) abolished the cytoprotective effect of the Nrf2 activator against H2O2. Inversely, it is also feasible that Sesn2 regulates the Nrf2 pathway. Transfection with Sesn2 induces degradation of Keap1, which results in Nrf2 accumulation in the cells. Sesn2-induced degradation of Keap1 is mediated by p62-dependant autophagy through its interaction with p62, Rbx1, and Keap1. Sesn2 ablation blocked Keap1 degradation and Nrf2 activation and thereby increased the susceptibility of the liver to oxidative damage.  

Hyperglycemia aberrantly increases degradation of glucose, which exerts harmful effects on the liver. Levels of methylglyoxal, an endogenous metabolite of glucose that increases in the blood and other tissues of patients with diabetes and exerts deleterious effects on cells and tissues. We recently reported that methylglyoxal induces hepatocyte death and liver toxicity, which results from oxidative stress and mitochondrial dysfunction. Sesn2 transfection inhibited methylglyoxal-induced mitochondrial dysfunction and apoptosis in hepatocytes. Moreover, mice infected with recombinant Sesn2 adenovirus repressed elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and attenuated GSH depletion induced by methylglyoxal in the liver. With prolonged endoplasmic reticulum (ER) stress, cells attenuate protein translation to prevent accumulation of misfolded proteins which is observed in many liver diseases. Sesn2 is induced by ER stress inducers such as tunicamycin or thapsigargin through the CCAAT-enhancer-binding protein beta. ER stress-inducing agent, nelfinavir, decreased mammalian target of rapamycin (mTOR) activity and increased Sesn2 expressions. Sesn2-knockout cells are susceptible to ER stress-mediated cell death and Sesn2-deficient mice developed severe ER stress-associated liver damage, steatohepatitis, and fibrosis. Collectively, Sesn2 activation is very useful for the prevention or treatment of oxidative stress-mediated liver injury.  

4.2. The Role of Sestrin2 in Hepatitis  
Hepatitis can be caused by infectious (e.g., viral, bacterial, fungal, and parasitic organisms) and noninfectious (e.g., alcohol, medications, autoimmune diseases, and metabolic diseases) factors. The liver contains many innate immune cells, such as natural killer and Kupffer cells (KC). Among them, KCs are considered important contributors to liver injury during hepatitis due to their pro-inflammatory activity. Activated KCs produce nitric oxide (NO), which results in oxidative stress through interaction with ROS, leading to the formation of peroxynitrite or upregulation of the expression of proinflammatory cytokines or chemokines. These inflammatory mediators directly regulate hepatocyte death or activate hepatic stellate cells, sinusoidal endothelial cells, or neutrophils. In addition, KCs express several toll-like receptors (TLRs) such as TLR 2–4, involved in the pathogenesis of hepatitis, and are the principal scavengers of intestinal microbial products such as lipopolysaccharide (LPS). We and others recently reported that Sesn2 induction by toll-like receptor ligands (TLRLs) or NO might protect against endotoxin stress in the liver. TLRLs treatment to macrophages induces Sesn2 expression and this induction was
due to increased transcriptional activity of activator protein-1 (AP-1) and Nrf2. Moreover, LPS treatment inhibited polyubiquitination and degradation of Sesn2. Especially, ectopic expression of Sesn2 through tail vein injection protected the liver against acute hepatitis induced by d-galactosamine (Gal)/LPS. Sesn2 expression reduced the serum ALT and AST levels induced by Gal/LPS.37 Gal/LPS treatment increased the degeneration of hepatocytes and the number of infiltrated inflammatory cells, which were attenuated by Sesn2. In addition, Sesn2 restored depleted GSH content and blocked inflammatory cytokine production (e.g., tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-1β) induced by Gal/LPS. Sesn2 overexpression blocked LPS-induced NO release and iNOS expression in macrophages. In addition, Sesn2 inhibited LPS-induced ROS production and cell death via inhibition of NADPH oxidase expression. Sesn2 inhibited LPS-mediated AP-1 activation, but not NF-κB.38 These results provide insight into the role of Sesn2 in innate immunity and inflammatory response.

4.3. The Role of Sestrin2 against Hepatic Metabolic Stress

The liver is a major organ to control nutrients, thus maintains metabolic homeostasis of organisms by regulating catabolic and anabolic processes.39,40 Compared to the bioenergetic needs of the organ, an insufficient supply of nutrients disrupts metabolic homeostasis; it results in hepatic metabolic stress leading to metabolic syndromes.41,42 Sestrin2 appears to protect cells against energetic stress-mediated apoptosis. Metabolic stress affects the activity of AMP activated protein kinase (AMPK), a stress sensor protein kinase, which downregulates mTOR, a master switch of anabolism and catabolism.40 To avoid an energy shortage, cells slow down their metabolism and inhibit anabolic reactions via the down-regulation of the mTOR pathway. Sesn2 negatively regulates mTOR signaling by activating AMPK and tuberous sclerosis 2 phosphorylation.43 Indeed, Sesn2 is upregulated in response to energetic stress such as 2-deoxyglucose and metformin and leads to the inhibition of mTOR.44 Sesn2 knockout mice fed with high fat diet (HFD) increased liver mTOR-S6K signaling.45 Treatment with AMPK activator (5-aminoimidazole-4-carboxamide) to HFD-fed Sesn2-knockout mice restored AMPK activity and suppressed mTORC1-S6K activity. In addition, Sesn2-knockout mice fed a HFD showed defective glucose homeostasis that was largely due to decreased hepatic insulin sensitivity.

Hepatic fat accumulation is associated with liver X receptor alpha (LXRα)-dependent signaling. LXRα activation results in hepatic steatosis induced by de novo fatty acid synthesis.46 We recently reported that Sesn2 transfection antagonized the ability of the LXRα-induced target gene transactivation and LXRα activity in hepatocytes.47 Additionally, the natural polyphenolic component, resveratrol, upregulated Sesn2 expression that resulted in repressed LXRα-activated sterol regulatory element binding protein-1c (SREBP-1c) expression and LXR- luciferase activity. These results suggested that Sesn2 might be an attractive target for the prevention and/or treatment of hepatic steatosis.

A recent study reported Sesn2 was upregulated in mice subjected to fasting or subsequent refeeding.51 Refeeding elicited an acute lipogenic response in the liver, as revealed by marked increases of lipogenic genes, such as SREBP-1c, fatty acid synthase, and stearoyl-coenzyme A desaturase 1, through mTORC1 activation, which led to fat accumulation and enhanced ROS production in hepatocytes. The Sesn2-knockout mice showed a substantial increase in liver damage against fasting and refeeding. Sesn2 regulates lipid homeostasis under ER stress, which have a significant impact on the pathogenesis of metabolic diseases.31 Palmitic or stearic acid elevates mRNA and protein levels of Sesn2, unlike unsaturated fatty acids. Several studies suggest that chronic ER stress inhibits mTOR signaling through an unveiled mechanism. Knockdown of Sesn2 failed to activate AMPK or inhibit mTORC1 signaling in response to high fat diet.

**Fig. 2. Sestrin2 as a Therapeutic Target to Prevent and Treat Liver Diseases**

Sestrin2 is involved in various functions, including hepatocyte injury, hepatitis, metabolic stress, and HCC; thus, it might be an advantageous target. Hence, we suggest that Sesn2 might be helpful in identifying valuable potential targets to prevent and/or treat liver diseases.
to saturated fatty acid or tunicamycin-induced ER stress, which demonstrated that mTOR suppression during ER stress is Sesn2-dependent. Sesn2-deficient obese mice exhibited exacerbated simple fat accumulation in the liver under ER stress conditions. Collectively, stress-inducible Sesn2 has an important homeostatic role in the control of lipid and glucose metabolism.

4.4. The Role of Sestrin2 in Liver Regeneration and Carcinogenesis Hepatocellular carcinoma (HCC) is one of the most lethal human malignancies. HCC frequently arises in the context of chronic liver injury, regeneration, and inflammation that promotes DNA damage and chromosomal aberrations. Sesn2 is a critical mediator of p53-mediated mTORC1 inhibition in cultured cells, as well as in the mouse livers.\(^{48}\) The ability of the hepatocarcinogen (e.g., diethylaminoethane) to inhibit S6 phosphorylation is Sesn2-dependent. Sesn2 increases its interaction with eIF-4E and inhibits expressions of growth regulatory proteins, such as cyclin D1 and c-Myc. Deletion of p21 was compensated by the activation of Sesn2, which subsequently impaired mTOR activity and activated cytoprotective Nrf2 signaling.\(^{49}\) It is suggested that the compensatory induction of Sesn2 by p21 loss not only inhibits mTOR signaling-mediated hepatocyte proliferation, but also increases the Nrf2-regulated oxidative stress response, thereby protecting mice against subsequent liver injury and tumor development.

5. CONCLUSION

Based on our current understanding of Sesn2, we know that Sesn2 plays a distinct role in liver pathophysiology. Sesn2 is associated with various cellular functions, including cell survival, inflammation, energy production and homeostasis, liver regeneration, and carcinogenesis. However, the exact mechanism of how Sesn2 reacts against physiological conditions in several liver diseases is still unclear; accumulated reports support the possibility of Sesn2 as a promising target in liver disease (Fig. 2). Further research is needed to determine the effects of Sesn2 on liver pathophysiology and understand fully the underlying regulatory mechanisms in more disease-specific contexts. Finally, in this review, we suggest that Sesn2 may be helpful in identifying valuable potential targets to prevent and/or treat liver diseases.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Woo HA, Bae SH, Park S, Rhee SG. Sestrin 2 is not a reductase for cysteine sulfenic acid of peroxiredoxins. Antioxid. Redox Signal., 11, 739–745 (2009).