ROS correlates intimately with the progression of non-alcoholic fatty liver disease to hepatocarcinoma

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The prevalence of non-alcoholic fatty liver disease (NAFLD) is rapidly increasing. NAFLD affects the health of one-third of the world’s population in forms that range from simple steatosis to hepatocellular injury, inflammation, fibrosis, liver cirrhosis and hepatocarcinoma. Oxidative stress in the progression of NAFLD to hepatocarcinoma is gaining increasing attention. Sustained and excessive reactive oxygen species (ROS), which lead to oxidative stress, are involved in all pathophysiological stages of NAFLD and contribute to the occurrence of hepatocarcinoma. Antioxidants in natural plant extracts that activate nuclear factor related to E2 factor 2 (Nrf2) can effectively suppress the progression. The cellular anti-oxidative system has great importance in the prevention and reversal of NAFLD. This review summarizes the current knowledge of the involvement of oxidative stress in NAFLD to hepatocarcinoma.

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Introduction

Reactive oxygen species (ROS), containing superoxide anion, hydroxyl radical and hydrogen peroxide, are small molecules that were originally thought to be produced by phagocytes as part of their role in host defence [1]. However, additional research has shown that ROS are generated by diverse sources in various cells of the body and are mainly produced as by-products of mitochondrial respiration [2] and by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases of the NOX family [3]. ROS act as important messengers in biological information transduction to affect downstream signalling systems. Redox homeostasis is very important to cellular physiological functions. Cells respond to endogenous and exogenous stimuli using a complex regulatory mechanism through which cell signalling systems involved in cell proliferation, differentiation, metabolism, ageing and other physiological processes are impacted by cellular redox status [4-6]. ROS regulate cellular responses through direct alteration of various kinases or transcription factors, such as adenyl cyclase, MAPK, and nuclear-factor xB (NF-xB), and indirect modulation of cysteine-rich redox-sensitive proteins [7]. Normal cells can maintain intracellular redox homeostasis. When cellular concentrations of ROS are elevated, cells initiate a defence system of anti-oxidation against ROS. The first components of this defence system are enzymatic scavengers (e.g., dismutases and peroxidases) and redox-sensitive modulators (e.g., vitamin E and glutathione) [8]. Then, ROS counteract the sequestration of nuclear factor related to E2 factor 2 (Nrf2) by Kelch-like ECH-associating protein 1 and provoke Nrf2 activation [9]. Nrf2 molecules enter the nuclei, bind
Antioxidant response elements in the regulatory regions of target genes, such as phase II detoxifying enzymes and antioxidant enzymes, and activate their transcription. Those enzymes then act to restore intracellular redox homeostasis and maintain normal physiological conditions (Fig.1). Nrf2 is highly important for maintaining redox homeostasis, especially in the liver. When ROS overwhelm the cellular antioxidant defense system, cellular oxidative stress would occur, through increase in ROS level or decrease of antioxidant capacity. This review summarizes the current findings of the involvement of oxidative stress in the progression of non-alcoholic fatty liver disease (NAFLD) to hepatocarcinoma (Fig.2 and Fig.3).

**ROS and NAFLD**

The prevalence of NAFLD is rapidly increasing recently. NAFLD is pathologically characterized by the accumulation of triglycerides in hepatocytes. And NAFLD has been associated with obesity, hyperlipidemia, insulin resistance, type 2 diabetes mellitus and metabolic syndrome. NAFLD presents with a spectrum of histological pathologies, from simple hepatocellular steatosis to hepatocellular injury with subsequent inflammation and fibrosis, then often results in liver cirrhosis and even hepatocarcinoma. NAFLD has been recognized as the most common liver disorder in developed countries, affecting over one-third of those countries’ populations. Although the underlying mechanisms are not completely understood, oxidative stress appears to be the major pathogenic event occurring through the duration of the disease. Previous studies also found significantly increased ROS in the liver of patients with viral hepatitis and alcoholic liver disease.

**Liver lipid accretion has increased significantly throughout the world because of calorie-enriched diet and lack of exercise.** Obesity is a major risk factor for hepatocellular steatosis. Free fatty acids and their metabolites play a key role in membrane structure, intracellular signalling and energy homeostasis. Excess lipids in the liver cause hepatic lipotoxicity that activates NADPH oxidase and disrupts mitochondrial respiration, and then, result in accumulation of ROS which induce oxidative stress. Mitochondrial dysfunctions involving ROS play a key role in the physiopathology of hepatic steatosis and precede the development of NAFLD. High dietary advanced glycation end-products exacerbate liver injury, inflammation and fibrosis via oxidative stress. Excess lipids initiate ER stress, and lipid-induced ROS can also initiate ER stress. The signalling pathway activated by ER stress has been linked to cellular perturbations that are common to obesity and NAFLD. Palmitate alters ER calcium stores and induces mitochondrial dysfunction characterized by elevated ROS accumulation and glutamine consumption. Liver steatosis is induced by carbon tetrachloride, which is involved in ROS production and P450 2E1 activation. ER stress is preferentially activated in patients who develop progressive steatohepatitis.

Approximately 10-30% of simple steatosis cases will evolve to non-alcoholic steatohepatitis (NASH), which is characterized by hepatocyte injury in the forms of ballooning and apoptosis, inflammation, necrosis and fibrosis. Oxidative stress is a strong contributor to the progression of steatosis to NASH. Kupffer cells, dendritic cells and lymphoid cells resident in liver parenchyma, have strong similarities and interconnections with innate immune cells and play major roles in the immune response to pathogens during the progression of steatosis to NASH. Cellular ROS released by hepatocytes induce nuclear-factor κB (NF-κB) activation in Kupffer cells, which promotes the synthesis of TNF-α and several pro-inflammatory cytokines, including interleukin-6 and interleukin-8. A variety of inflammatory mediators are released, and local inflammation occurs. Rats treated with carbon tetrachloride showed significantly increased mRNA expression in liver and serum protein levels of TNF-α, interleukin–6 and interleukin–1β. The aim of the inflammatory response is to remove external damaging factors, contributing to tissue repair and promoting the re-establishment of homeostasis; however, excessive inflammation may induce a massive loss of...
hepatocytes and, hence, exacerbate the severity of various hepatic conditions. When hepatic stellate cells (HSCs) are activated, local liver fibrosis occurs. The aim of the local formation of fibrosis is to limit the spread of pathogens and repair local tissue. Tissue inhibitor metalloproteases are produced at the same time to degrade collagen. The liver maintains its normal structure if the synthesis and degradation of collagen are balanced. The coordinate regulation by hepatocytes and mesenchymal cells for liver reparation is a self-protection mechanism of the liver to maintain its physiological structure and function. Once hepatocellular injury, inflammation and fibrosis become uncontrollable, liver damage occurs.

Scholars have found many antioxidants in natural plant extracts that eliminate oxidative stress and can cure NAFLD and inhibit steatosis from developing into NASH. Punicalagin significantly inhibits high fat diet induced hyperlipidaemia and hepatic lipid deposition by promoting mitochondrial function and, thus, eliminating oxidative stress. Chlorogenic acid efficiently inhibits LPS-induced pro-inflammatory responses by depressing the LPS/ROS/NF-κB signalling pathway in HSCs. Consumption of hydrogen-rich water is an effective treatment for NASH, as it reduces hepatic oxidative stress, apoptosis, inflammation, and hepatocarcinogenesis. Targeting oxidative stress might be valuable for NASH treatment.

**ROS and liver fibrosis developing into cirrhosis**

Approximately 20-30% of NASH patients will develop...
progressive liver fibrosis and, ultimately, cirrhosis. HSC is considered the most relevant pro-fibrogenic cell type involved in this process [56]. ROS can induce HSCs to undergo a phenotypic switch from quiescent cells to myofibroblast-like cells, which exhibit upregulated collagen synthesis. Excessive ROS generation can directly activate HSC through chloride channels if damage to hepatocytes persists [57] and promote the secretion of platelet derived growth factor (PDGF) [58] and transforming growth factor-β1 [59]. PDGF activates NADPH oxidase in HSCs to generate more ROS and further promote the proliferation of HSCs and the progression of fibrosis [60-62], and p38MAPK phosphorylation is involved in this process [62]. When this occurs, the redox homeostasis in the liver is destroyed and liver fibrosis spreads throughout the entire liver. A growing number of studies have found that redox balance disorder is important in HSC activation, proliferation, scalability and fibre phenotypic expression [63, 64]. Inhibition of ROS during cholestasis can reduce liver fibrosis [65].

Curcumin, silybin-phytosome [66] and salvianolic acid are antioxidants and can inhibit liver fibrosis [61]. Neferine was found to inhibit cultured HSC-T6 cell activation and induce apoptosis by increasing Bax and caspase-3 expression via the mitochondrial pathway [67]. An increasing number of studies have found that antioxidants in natural plant extracts activate Nrf2, indicating that antioxidants can suppress liver fibrosis progression [67-70].

ROS and hepatocarcinoma

Hepatocarcinoma is the consequence of chronic liver disease, especially NAFLD. Sustained and excessive ROS are involved in all pathophysiological stages that contribute to the occurrence of hepatocarcinoma. Oxidative DNA damage is a significant source of genomic instability [71], ROS influence gene expression through the regulation of transcription factors [72]. Cells can adapt defence systems against the deleterious effects of ROS. Tumours develop when cellular defence and repair systems are defective [73]. ROS induce epigenetic modulations in the process of hepatocyte carcinogenesis [74]. Oxidative stress induces an amino acid substitution in growth inhibitor 1, a tumour suppressor that is down-regulated or altered in human hepatocellular carcinoma (HCC) [75]. NADPH quinine oxidoreductase 1 (NQO1) plays a crucial role in the protection against oxidative stress. Individuals carrying the NQO1 variant allele and genotypes are more susceptible to HCC [76]. Oxidative stress promotes the proliferation of preneoplastic lesions and their progression to neoplasms [77]. HCC development is accompanied by a continuous increase in ROS levels [78-81]. NF-κB is activated by ROS and subsequently enhances the migration of HCC [82, 83]. Sustained oxidative stress acts in multi-stage carcinogenesis [84, 85].

At present, many studies have shown that antioxidants can suppress the occurrence and development of hepatocarcinoma. Curcuma was shown to protect mice with hepatic injury from inflammatory and oxidative stress. Curcuma oil can inhibit hepatoma cell growth in vivo and in vitro [86]. Dietary phytochemicals, such as phenolic acids, monophenol, and polyphenol, and their derivatives inhibit cancer invasion and metastasis [87,88]. Defence enzymes mediated by Nrf2 can contribute to cellular protection against ROS and reactive metabolites of carcinogens [89].

Conclusions

ROS are involved in all stages of the pathophysiological process of NAFLD to hepatocarcinoma [90-92], including hepatocellular steatosis, subsequent inflammation [93], fibrosis, liver cirrhosis [94,95], and occurrence of hepatocarcinoma [84, 96]. Many cytokines and different cell types participate in the chronic injury progression involved in complicated regulatory networks and crosstalk [97-100]. The liver also performs antioxidant and defensive protection mechanisms during oxidative stress. During chronic injury progression, injury and repair act against each other. Lesions appear when the injury inflicted is greater than the repair performed [101], and liver remains normal when the repair performed is greater than the injury inflicted. Researchers have shown that the antioxidant enzyme glutathione can scavenge ROS in hepatocytes [102]. Antioxidants can suppress the occurrence and development of hepatocarcinoma [13, 87-89, 103]. The cellular anti-oxidative system has great importance in the prevention and reversal of chronic injury in liver. An increasing number of researchers have placed attention on the possibility of using antioxidant therapy for NAFLD to
hepatocarcinoma. Inhibiting hepatocellular oxidative stress to maintain redox homeostasis is likely the key step in maintaining the health of the liver. For patients of NAFLD, quicker intervention to maintain redox homeostasis in the liver may have a greater benefit to prevent the occurrence of hepatocarcinoma.

**Conflicting interests**

The authors have declared that no competing interests exist.

**Abbreviations**

HCC: human hepatocellular carcinoma; HSCs: hepatic stellate cells; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; Nrf2: nuclear factor related to E2 factor 2; NF-κB: nuclear-factor κB; NADPH: nicotinamide adenine dinucleotide phosphate; NQO1: NADPH quinine oxidoreductase 1; PDGF: platelet derived growth factor; ROS: reactive oxygen species.

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