REVIEW ARTICLE

Oxidative stress in obesity-associated hepatocellular carcinoma: sources, signaling and therapeutic challenges

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Obesity affects more than 650 million individuals worldwide and is a well-established risk factor for the development of hepatocellular carcinoma (HCC). Oxidative stress can be considered as a bona fide tumor promoter, contributing to the initiation and progression of liver cancer. Indeed, one of the key events involved in HCC progression is excessive levels of reactive oxygen species (ROS) resulting from the fatty acid influx and chronic inflammation. This review provides insights into the different intracellular sources of obesity-induced ROS and molecular mechanisms responsible for hepatic tumorigenesis. In addition, we highlight recent findings pointing to the role of the dysregulated activity of BCL-2 proteins and protein tyrosine phosphatases (PTPs) in the generation of hepatic oxidative stress and ROS-mediated dysfunctional signaling, respectively. Finally, we discuss the potential and challenges of novel nanotechnology strategies to prevent ROS formation in obesity-associated HCC.

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INTRODUCTION

Liver cancer is the sixth most common diagnosed cancer and the third cause of cancer death worldwide [1]. Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancers and is refractory to nearly all currently available anticancer therapies with a 5-year survival rate of nearly 15% [2]. Over the last 20 years, the incidence of HCC has been rapidly increasing in economically developed nations and is mostly attributable to nonalcoholic fatty liver disease (NAFLD) [2]. The obesity epidemic is thought to now account for as much as 40% of the increase in HCC in developed countries [3]. The hallmark of NAFLD is the accumulation of fat in hepatocytes (i.e., steatosis). Indeed, the main risk factors of NAFLD are obesity and type 2 diabetes (steatosis occurs in >75% of all obese individuals and prevalence increases by a factor of 4.6 for BMI ≥30 kg/m^2), which can progress to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and HCC [2, 3] (Fig. 1). There are ~1.9 billion overweight adults (BMI >25 kg/m^2) worldwide of whom >650 million are obese (BMI >30 kg/m^2). This is predicted to rise in the near future and to be largely unabated by lifestyle intervention (World Health Organization). While HCC development is more common in patients with cirrhosis, obese subjects with NASH can develop HCC without fibrosis [4], suggesting that the relationships between steatosis, NASH, fibrosis/cirrhosis, and HCC are not necessarily linear (Fig. 1).

Many efforts have been made in the past years to understand how hepatic fat accumulation associated with obesity leads to a tumor-promoting environment in the liver. Several molecular details remain to be fully elucidated, but accumulating evidence shows that HCC can develop as a consequence of local and systemic inflammation, like other types of cancers [5]. Inflammation is a physiological and complex defensive response of the body against endogenous or exogenous injurious triggers. During obesity, this response is associated with the expansion of the adipose tissues and ectopic fat accumulation in the liver. Immune cells are recruited to these organs in an attempt to restore the homeostasis, but if fat overload persists the inflammation becomes chronic and gradually increases liver damage affecting key signaling pathways and leads to the progression from simple obesity-associated hepatic steatosis to more advanced stages of NAFLD, including HCC [4, 5].

Hepatocyte exposure to excessive levels of lipids stimulates oxidative stress and cell damage through different mechanisms [6]. The toxic effects of lipids are frequently referred to as lipotoxicity. Because the liver has a high regenerative capacity, oxidative stress and eventual hepatocyte death induce substantial cell proliferation and simultaneously induce the expansion of hepatic resident Kupffer cells [7]. The activated Kupffer cells secrete cytokines and chemokines that regulate key signaling pathways and recruit other immune cells, intensifying inflammation and further stimulating reactive oxygen species (ROS) production. In parallel, chronic oxidative stress increases the frequency of genomic DNA mutations and can affect the expression of HCC development-related genes [7]. Moreover, in the context of obesity, hepatic inflammation and oxidative stress occur not only as a response to events originating within the liver itself but also as a consequence of signals derived from distant tissues, including the intestines with the altered microbiome composition and white adipose tissues with altered adipokine production and secretion [4, 5, 7]. Synergically or independently, these events can all promote liver carcinogenesis and cancer

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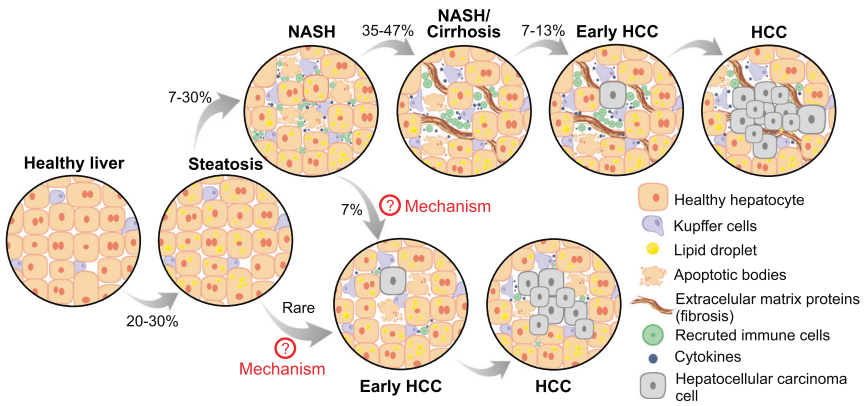

Fig. 1 Progression from nonalcoholic fatty liver disease (NAFLD) to nonalcoholic steatohepatitis (NASH), fibrosis, and HCC. NAFLD is characterized by excessive lipid accumulation in lipid droplets in the cytosol of hepatocytes. Lipotoxicity causes hepatocyte death and activation and proliferation of Kupfer cells, as well as recruitment of other immune cells to the liver causing inflammation and accelerating the progression from simple steatosis to NASH. The inflammation and tissue damage lead to pathological wound healing with an accumulation of extracellular matrix proteins characterizing the fibrosis/cirrhosis, which is usually accompanied by changes in the microenvironment of the liver affecting the genetics and cellular signaling, favoring the emergence of hepatocellular carcinoma cells and development of small tumors at the early developmental stage of HCC. Without intervention, early HCC progresses to more advanced stages of HCC. About 7% of the patients with NAFLD/NASH can develop HCC without cirrhosis, but the mechanism of this progression is currently unknown.

progression in obese individuals. Taken together, there is strong evidence that fatty liver is an important risk factor for HCC and deciphering the mechanisms behind the complex networks caused by oxidative stress-entailed dysfunctional hepatocytes is therapeutically relevant. Here, such an intricate relationship is reviewed, with a particular emphasis on recent advances regarding hepatic changes caused by oxidative stress, in pathways associated with hepatocarcinogenesis in NAFLD.

DIFFERENT SOURCES OF OXIDATIVE STRESS AND THEIR CONTRIBUTIONS TO HCC

Accumulating evidence suggests that increased oxidative stress contributes to the development of liver cancer [8]. Oxidative stress is caused by the increased production of ROS and reactive nitrogen species. They consist of superoxide radical ($^{\cdot}O_2^-$), nitric oxide radical ($^{\cdot}NO$), hydroxyl radical ($^{\cdot}OH^-$), and uncharged species such as hydrogen peroxide (H_2O_2) [9]. Although initially described as toxic agents, it is now clear that these molecules serve as regulators of cellular homeostasis and participate in several physiological processes necessary for cell survival and function [9]. In pathophysiology, however, increased ROS production triggers oxidative damage of proteins, DNA, and lipids. Depending on their source (cell or tissue type), level of production, and surrounding cellular environment, ROS contribute to physiological processes or the development of pathological signaling. ROS are typically produced as a result of various enzymatic reactions in different compartments of a cell, namely cytosol, mitochondria, peroxisomes, and endoplasmic reticulum (ER) [9] (Fig. 2). These organelles can also act as scavengers by the action of enzymatic and nonenzymatic antioxidant systems to prevent oxidative damage. Any imbalance between the inter-organellar contributions to ROS production and detoxification can have consequences on the entire cell physiology and pathophysiology.

Cytosolic ROS

Cytosolic ROS are mainly formed by the activity of a family of NADPH oxidase (NOX) enzymes [10]. NOX is a membrane-bound enzyme complex that generates $^{\cdot}O_2^-$ by transferring one electron to O_2 from NADPH and has been implicated in several metabolic processes including glycolysis, oxidative phosphorylation, and pentose phosphate pathway [10]. NOX1, NOX2, and NOX4 have been shown as related to the activation of hepatic stellate cells and hepatocyte cell death, the essential steps for initiation of liver fibrosis, and progression to HCC [11].

Mitochondrial ROS

Mitochondrial dysfunction is the main source of hepatic oxidative stress derived from energetic metabolism due to lipotoxicity and inflammation [12]. Mitochondria utilize O_2 to produce ATP from substrate catabolism (free fatty acids, glucose, and ketone bodies) however, a small percentage of O_2 is converted to $^{\cdot}O_2^-$, which can be modified to other forms of ROS. Superoxides are produced as part of normal mitochondrial respiration and they are typically scavenged by a family of mitochondrial superoxide dismutase (SOD) proteins to H_2O_2 (by SOD1 isoform when $^{\cdot}O_2^-$ is produced in the inter membrane mitochondrial space and by SOD2 isoform when $^{\cdot}O_2^-$ is produced in the matrix) [10]. H_2O_2 is further converted to H_2O and O_2 by glutathione peroxidase (GPX). Mitochondrial ROS promote neoplastic transformation in different tissues and are associated with HCC progression in obese patients. High levels of circulating fatty acids increase levels of hepatic free fatty acids, which are metabolized by mitochondria in the β-oxidation pathway [12]. Under oxidative stress, NAD^+ and FAD are reduced into NADH and $FADH_2$ and deliver electrons to the respiratory chain. An imbalance between increased electron delivery and reduced electron outflow from the respiratory chain causes electrons and ROS products to accumulate [12]. ROS impair mitochondrial function by multiple mechanisms. First, the interaction of ROS with lipids (mostly polyunsaturated fatty acids) leads to the formation of lipid peroxidation products such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA), contributing to NASH progression [13]. Second, lipid peroxidation products target mitochondrial DNA (mtDNA, encoding 13 proteins involved in the electron transport chain function) leading to mitochondrial dysfunction, which is linked to inflammation and NASH [14]. Due to the proximity to the electron transport chain, lack of protective histones, and incomplete DNA repair mechanisms, mtDNA is susceptible to ROS-mediated oxidative damage. mtDNA damage impairs mitochondrial respiratory chain function, which further results in ROS and subsequent accumulation of damaged mtDNA. Collectively, ROS-induced damage triggers a vicious cycle, where excessive ROS are produced due to mitochondrial dysfunction, which in turn can induce further oxidative detrimental effects to mitochondrial structure and function.

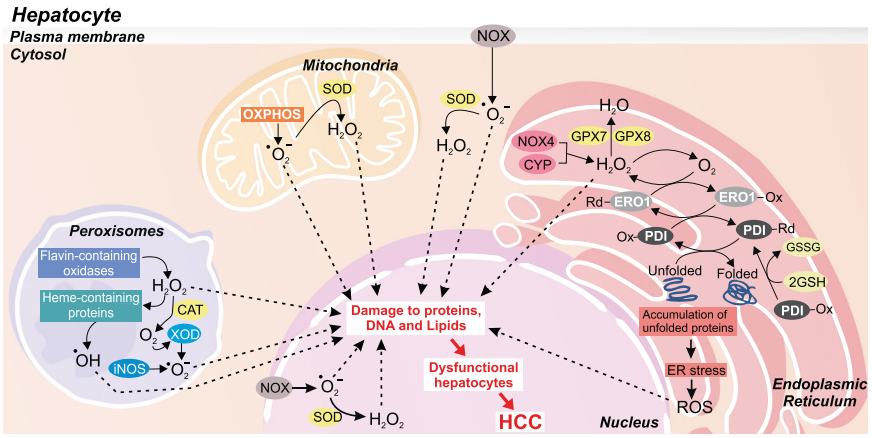

Fig. 2 ROS production in different cell compartments and their contributions to HCC development. Nicotinamide adenine dinucleotide phosphate oxidases (NOX) are membrane-bound enzymes that generates $^{\cdot}O_2^-$ in the cytosol. Mitochondria utilize oxygen to produce ATP during oxidative phosphorylation (OXPHOS) and a percentage of oxygen is converted to $^{\cdot}O_2^-$, which is typically scavenged by a family of mitochondrial superoxide dismutase (SOD) to H_2O_2. In peroxisome, flavin-containing oxidases transfer electrons from various metabolites and reduce oxygen to H_2O_2. This class of enzymes includes acyl-CoA oxidases (ACOX) and xanthine oxidoreductase (XOD). Under certain posttranslational modifications, XOD also reduces O_2 to $^{\cdot}O_2^-$. The nitric oxide synthase (iNOS) is also present in peroxisomes of hepatocytes and can generate $^{\cdot}O_2^-$. Besides, the peroxisomes environment is rich in heme-containing proteins that produce $^{\cdot}OH$ from H_2O_2 by the Fenton reaction. In the endoplasmic reticulum (ER) the oxidative protein folding process involves enzymes as protein disulfide isomerase (PDI) and ER oxidoreductin 1 (ERO1) that produces H_2O_2. NADPH oxidase 4 (NOX4) and cytochrome P450 (CYP) are also present in the ER, representing other sources of H_2O_2, which is then scavenged by peroxidases GPX7 and GPX8. Also in the ER, the accumulation of unfolded proteins leads to ER stress and stimulates ROS production. In the nucleus, NOX is also a $^{\cdot}O_2^-$ source, which can be scavenged by SOD to H_2O_2. Excessive production of ROS in these different cell compartments can overwhelm the antioxidant systems, causing oxidative damage to different cellular components, affecting the cell functions, and can eventually lead to carcinogenesis.

Peroxisome

The peroxisome is an organelle particularly abundant in liver cells. Peroxisomes are important cellular organelles regulating the aerobic metabolism of lipids, but they also possess a parallel role in producing ROS. In contrast to mitochondria, the respiratory pathway in peroxisomes does not yield ATP, but electron transfer from various metabolites reduces oxygen to H_2O_2 which is further detoxified by catalase enzyme activity (GPX, in mitochondria) [15]. The flavin-containing oxidases are the most abundant class of H_2O_2-producing enzymes inside peroxisomes. This class of enzymes includes acyl-CoA oxidases (ACOX) involved in the peroxisomal β-oxidation of very long and branched-chain fatty acids and xanthine oxidoreductase (XOD) required for the catabolism of purines [16]. Under certain posttranslational modifications, XOD also acts as an oxidase that reduces O_2 to $^{\cdot}O_2^-$ [17]. Another source of prooxidant molecules is the inducible nitric oxide synthase (iNOS) found in hepatocyte's peroxisomes mostly in the monomeric form and which generates $^{\cdot}O_2^-$ [18]. A proportion of the pool of iNOS induced under inflammation is targeted to peroxisomes and can result in catalase downregulation [19]. In addition, the peroxisomes are rich in heme-containing proteins, and in this environment, H_2O_2 can produce $^{\cdot}OH$ through the Fenton reaction. These sources of H_2O_2 and free radicals require competent antioxidant defense systems and the unbalance between them can represent a key cause of oxidative stress and HCC development [16].

Endoplasmic reticulum

The ER is a complex and large cellular organelle related to protein translation, translocation, posttranslational modification, and folding, and is involved in lipid and steroid hormone synthesis and calcium homeostasis [20]. Both the rough and smooth ER are abundant in hepatocytes. Similar to mitochondria and peroxisomes, ER can also represent a source of ROS and have an antioxidant system [21]. Folding of nascent proteins at the ER requires the introduction of a disulfide bond, necessary for protein stabilization. During this process, electrons from protein disulfide isomerase (PDI) are used to reduce ER oxidoreductin 1 (ERO1), an enzyme that acts as a source of oxidizing equivalents in the ER. Reduced ERO1 then converts molecular O_2 to H_2O_2 (Fig. 2). Reduced glutathione (GSH) assists in reducing non-native disulfide bonds in misfolded proteins, resulting in the production of oxidized glutathione (GSSG). The GSH/GSSG ratio in the ER is low compared to the cytosol, and this oxidative environment is necessary to maintain proper oxidative protein folding in the ER. H_2O_2 is also formed by NOX4 in the ER [22] and then scavenged by peroxidases GPX7 and GPX8. Accumulation of unfolded proteins can trigger ER stress, subsequently enhancing ROS formation and oxidative stress [20]. Thus, the oxidative protein folding process is enhanced to restore the proper protein structure, further generating H_2O_2 and depleting GSH. This response increases the levels of oxidative stress, which enhances ER stress. If not resolved, the vicious cycle leads to overall disruption of the ER function and cell death. Numerous pathophysiological changes are associated with ER stress, including obesity-induced HCC [23].

Genetic associations

Cirrhosis is a strong risk factor for hepatocarcinogenesis. Thus, oxidative stress can impact HCC occurrence by promoting fibrosis accumulation. The association between oxidative stress in NAFLD has been underlined by candidate genes studies that identified variants in *SOD2* and uncoupling protein 2 (*UCP2*) [24, 25]. Most of these studies had a modest sample size and have not been independently replicated. More recently, large genome-wide association studies have detected rs2642438 (p.A165T), a rare missense variant in the mitochondrial amidoxime reducing component 1 (*MARC1*) gene that reduced the risk of NAFLD-related cirrhosis [26]. While the exact function of *MARC1* is unknown, this protective variant might hamper ROS production by reducing nitric oxide production [27] and detoxify trimethylamine *N*-oxide [28]. However, the underlying mechanism which may contribute to cirrhosis is not yet understood.

To date, no genome-wide association studies have specifically assessed the relation between genetic variations in NAFLD, NASH, and HCC. However, candidate genes studies have linked oxidative

stress to liver carcinogenesis. A meta-analysis of 23 studies, mostly conducted in Asians, identified variants in glutathione *S*-transferase Mu 1 (*GSTM1*) and theta 1 (*GSTT1*) genes [29]. In cirrhotic patients of European ancestry, an association between variants in *SOD2*, myeloperoxidase (*MPO*)—expressed in neutrophils and Kupffer cells [30]—and HCC has also been reported [31]. Increased ROS due to mitochondrial dysfunction could also cause direct nuclear DNA damage [32], or impair repair mechanisms and ultimately favor alterations in oncogenes and tumor suppressor genes [33]. For example, somatic mutations activating nuclear factor erythroid 2-related factor-2 (*NFE2L2*) or inactivating Kelch-like ECH-associated protein 1 (*KEAP1*) have been reported in 5 to 15% of HCC [34, 35].

Metabolic dysfunction

It has been shown that high-fat content in the diet can alter glucose metabolism in normal liver cells to a Warburg-like phenotype, suggesting that excessive fat availability can metabolically prime normal hepatocytes for neoplastic transformation. The hyperactivation of glucose metabolism induced by high-fat availability is mediated by increased peroxisomal ROS production [36], highlighting the close link between energy metabolism and modifications in the oxidative status of the cells. In this sense, the availability of different nutrients and metabolites can influence not only the activity and the expression of anti- and prooxidant enzymes, but also feed metabolic pathways that burst ROS generation, driving metabolic reprograming, and carcinogenesis. For example, a liver-specific inhibitor of acetyl-CoA carboxylase (ACC), an enzyme producing intermediate metabolite for de novo lipogenesis, prevents HCC development [37]. Similarly, another study using multiomics analysis in vivo demonstrated that the mammalian target of rapamycin (mTOR) promotes hepatic de novo lipogenesis leading to hepatic steatosis and HCC [38]. These reports indicate that increased lipid storage drives HCC.

A growing body of recent evidence suggests the role of gluconeogenesis (glucose formation from noncarbohydrate precursors) in influencing HCC development. Gluconeogenesis is an essential metabolic process in hepatocytes and the limiting step of this pathway is catalyzed by the phosphoenolpyruvate carboxykinase (PEPCK) enzyme. During HCC, hepatic gluconeogenesis is suppressed due to both reduced PEPCK expression [39] and sumoylation-mediated inhibition of PEPCK1 [40]. Interestingly, increased gluconeogenesis is detrimental to malignant hepatocytes in HCC by increasing ROS [39], suggesting that restoring the reduced gluconeogenesis may prove to be a potential treatment strategy for HCC.

Inflammation

The cell-mediated inflammatory response involved in the propagation of inflammation as NAFLD progresses from simple steatosis to NASH results from damages induced by toxic lipids, oxidative stress, and the release of signals to recruit and activate immune cells in the liver. When active, some immune cells can produce large quantities of ROS and exacerbate liver damage, increasing cell turnover, hepatic stellate cell activation, fibrogenesis, and eventually cirrhosis, raising the risk for HCC. In contrast, the direct progression from NAFLD/NASH to HCC without fibrosis in a subset of HCC patients indicates important genetic and epigenetic influences on individual predisposition to hepatocarcinogenesis in obese conditions (see above). The liver environment in simple steatosis and NASH shares excessive fat accumulation and oxidative stress as common features, emphasizing their importance for HCC. Most patients with NAFLD-associated HCC have a NASH/cirrhosis history, implying that the peculiarities of inflammation and fibrosis are major components for HCC development, although not always essential.

The fibrogenic and inflammatory responses in the liver can be mediated by the activation of inflammasomes, multimeric cytosolic protein complexes that serve as pattern recognition receptors. They intersect with a wide variety of immune and cell death pathways. The component of the inflammasomes that acts as a sensor molecule is usually a member of the nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) family. NOD-like receptor pyrin domain-containing 3 (NLRP3) is the most studied member of this family known to sense a broad range of stimuli. NLRP3 inflammasome activation governs the cleavage and activation of Caspase-1, resulting in the maturation of pro-inflammatory cytokines, such as pro-interleukin IL-1β and pro-IL-18 [41]. NLRP3 inflammasome activation has been recognized to play a major role during the progression of NAFLD by causing severe liver inflammation and fibrosis and leads to pyroptotic cell death [41]. In the context of obesity, some danger signals, such as saturated fatty acids, ceramides, and cholesterol crystals promote activation of the NLRP3 inflammasome. The mechanism of palmitate-mediated NLRP3 inflammasome activation is reported to depend on the accumulation of dysfunctional mitochondria leading to increased mitochondrial ROS generation, suppression of the activation of AMP-activated protein kinase (AMPK), and autophagy signaling cascades [42]. NLRP3 inflammasome components are downregulated in human HCC, which is correlated with advanced stages and poor pathological differentiation, showing that the malfunction of the inflammasome in sensing danger signals may result in liver cancer with the aggressive phenotype [43].

In summary, oxidative stress-induced hepatic carcinogenesis is a complex and heterogeneous process, involving different organelles and multiple dysregulated proteins. The specific pathways affected seem to be highly dependent on the type, strength, and duration of the enhanced ROS formation. It is also likely that multiple pathways operate within the same and different hepatic cells, with initiating components being determined by the genetic and environmental factors (e.g., diets rich in saturated fats and alcohol consumption). In support of coordinate activation of multiple redox systems in the hepatic cells, there is no evidence of one component whose inhibition or deletion completely abrogates obesity-induced HCC formation.

CLASSIC ROS-ASSOCIATED SIGNALING IN HCC DEVELOPMENT

Oxidative stress in physiology and liver dysfunction

ROS signaling modulates a myriad of cellular targets ranging from proteins and genes to enzyme activity and transcription [9]. However, while excessive ROS production has been linked to many human pathologies, including insulin resistance in obesity, evidence suggests that low ROS levels are indispensable for normal cellular signaling. Thus, mouse models with the deletion of a key ROS scavenging enzyme (GPX1) demonstrated that ROS can offer protection against insulin resistance [44]. Interestingly, NOX4 has been shown to be highly expressed in healthy liver cells [44, 45], indicating that physiological ROS are important for normal hepatic signaling. ROS are necessary for both maintaining insulin sensitivity and promoting insulin resistance in metabolic tissues. Thus, the question remains—how ROS signaling exhibit its dual role in insulin sensitivity? To discern this dichotomy, it has been postulated that amount, types, the timing of ROS generation, and downstream signaling define the cell outcome. ROS in physiology and in the early phase of the disease (nutritional overload state such as high-fat diet) are beneficial for insulin signaling, but sustained high levels of oxidative stress lead to insulin resistance and liver dysfunction [46]. We will next describe classical signaling cascades targeted by ROS and their impact on liver dysfunction and cancer development (Fig. 3).

The role of NRF2

The most well-established signaling cascade regulated by ROS is mediated by the nuclear factor (erythroid-derived 2)–related

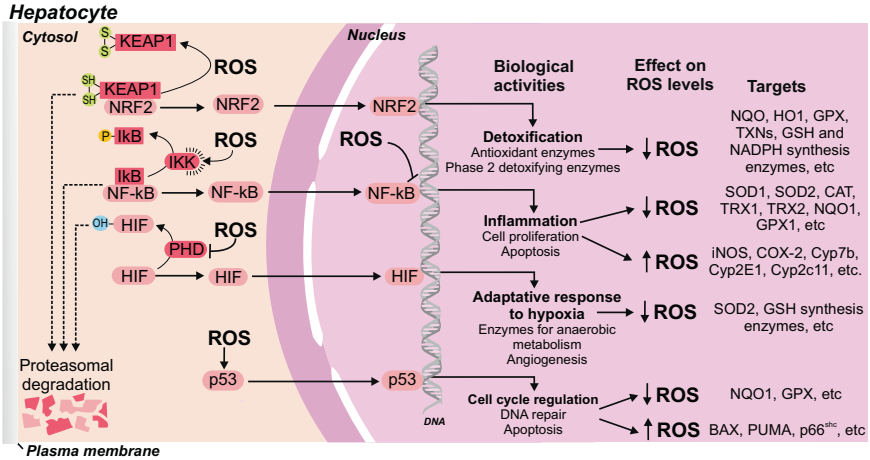

Fig. 3 Redox-regulated transcription factors. Reactive oxygen species (ROS) modulate the activation of several transcription factors involved with hepatocellular carcinoma development by indirectly interacting with proteins that regulate the activity of the transcription factors (NRF2, NF-kB, and HIF) or by directly reacting with the transcription factor (p53). These redox-regulated transcription factors affect diverse biological activities, including ROS and xenobiotic detoxification, inflammation, cell proliferation, apoptosis, adaptation to hypoxia, metabolism, angiogenesis, cell cycle regulation, and DNA repair. Some of these biological activities are accompanied by changes in the ROS levels, mediated by changes in the expression of different antioxidant and prooxidant enzymes and enzymes involved in the synthesis of molecules involved in the oxidative status of the cells, as GSH and NADPH. KEAP1 kelch-like ECH-associated protein 1, NRF2 nuclear factor (erythroid-derived 2)–related factor-2, NF-κB nuclear factor kappa B, IkB NF-κB inhibitor, IKK NF-κB inhibitor kinase, HIF hypoxia-inducible factor, PHD prolyl hydroxylase, p53 tumor protein p53, NQO1 NAD(P)H quinone dehydrogenase 1, HO1 heme oxygenase 1, GPX glutathione peroxidase, TXN thioredoxin, GSH reduced glutathione, NADPH nicotinamide adenine dinucleotide phosphate, SOD superoxide dismutase, CAT catalase, TRX 1/2 thioredoxin 1/2, iNOS inducible nitric oxide synthase, COX-2 cyclooxygenase 2, Cyp7b 25-hydroxycholesterol 7-alpha-hydroxylase, Cyp2E1 cytochrome P450 2E1, Cyp2c11 cytochrome P450, subfamily 2, polypeptide 11, BAX BCL-2-like protein 4, PUMA p53 upregulated modulator of apoptosis, $p66^{shc}$ Src homology/collagen (Shc) adapter protein.

factor-2 (NRF2). Under normal conditions, NRF2 translocation to the nucleus is prevented by the interaction with its binding partner KEAP1 and consequent proteasomal degradation [47]. Under oxidative stress, KEAP1 is oxidized in several cysteine residues, leading to protein conformational changes and dissociation from NRF2. This dissociation allows NRF2 translocation into the nucleus, where it binds to the antioxidant response element (ARE) in the DNA to transcriptionally increases the expression of enzymes as NQO, HO1, GPXs, TXNs, enzymes involved in GSH synthesis, and NADPH production as part of a response to protect against oxidative stress and xenobiotics [47]. NRF2 can either suppress or promote hepatocarcinogenesis, depending on the stage in which its activation occurs. The neoplastic transformation can be prevented when the NRF2 pathway is active soon enough to avoid chronic oxidative damage. On the other hand, when active in neoplastic cells, NRF2 signaling restricts ROS damage and favors cancer cell survival under chronic oxidative stress. It is believed that some cancer cells actually use NRF2 signaling as an adaptive mechanism to promote tumor growth [47]. Recently it has been proposed that NRF2 activation due to the microenvironment could be one of two hits required for HCC, while a mutation in the gene encoding β-catenin, a major genetic aberration observed in a significant subset of HCC, provides the second hit [48].

The role of nuclear factor-kappa B (NF-κB)

NF-κB mediates cellular responses to pro-inflammatory cytokines. ROS can either activate or repress NF-κB activity depending on the cell compartment (cytosol or nucleus). Once active, the NF-κB pathway can have both pro- and antioxidant roles by affecting the expression of target enzymes involved in ROS scavenging and generation, depending on physiological circumstances and cell type. This duality of responses highlights the close relationship between NF-κB and the redox status of the cells. In the cytosol, H_2O_2 can oxidize and activate NF-κB inhibitor kinase (IKK). IKK phosphorylates NF-κB inhibitor (IκB) that dissociates from NF-κB. Phosphorylated IκB is subjected to proteasomal degradation while NF-κB translocates to the nucleus and binds to DNA to promote the transcription of inflammatory and antiapoptotic genes. However, nuclear H_2O_2 can suppress NF-κB DNA binding, inhibiting its transcriptional activity [9]. Similar to NRF2, the timing of NF-κB activation is important to define its activity for carcinogenesis and tumor growth. Despite the role of NF-κB in maintaining antioxidant defenses and reducing liver damage [49], obese and NAFLD patients display increased pro-inflammatory cytokine levels, hepatic NF-κB activation and risk for HCC development [50].

The role of p53

ROS can indirectly and directly affect the tumor suppressor p53 activity. The direct action may depend on the oxidation of cysteine residues of p53 with potential consequences for its stability and transcriptional activity [51]. Reciprocally, p53 maintains the cellular redox balance by regulating the expression of pro and antioxidant proteins, including NQO1, GPX, BAX, PUMA, and $p66^{shc}$ [9, 52]. There is evidence that p53 activation is part of the pathogenesis and progression of HCC in obesity: p53 expression or signaling is increased in the livers of NAFLD patients or experimental NASH and p53 knockout mice are protected against methionine-choline deficient diet-induced oxidative stress and hepatic injury [53]; p53 inhibition protects against high-fat diet-induced hepatic steatosis in mice [54]; p53 gene mutation was found in HCC patients with a history of NAFLD [55]. The specific role of p53 in the link between NAFLD and HCC is thought to depend on the intensity of p53 activation because a gradual increase of p53 seems to protect against NAFLD progression, while higher expression causes liver inflammation and NAFLD progression.

The role of hypoxia-inducible factors (HIF)

HIF are transcription factors essential for cell survival in hypoxic conditions. HIF prolyl hydroxylases (PHD) hydroxylate HIF proteins in an O_2 dependent manner, leading to HIF proteasomal degradation. During hypoxia, HIF accumulates and translocates into the nucleus and transcriptionally activates genes required for

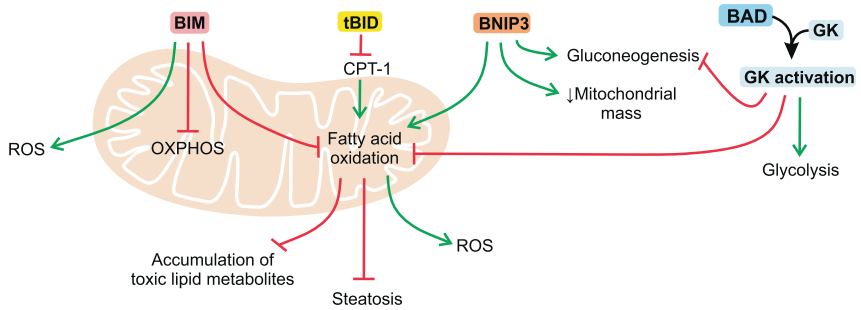

Fig. 4 BCL-2 family proteins are components of programmed cell death that can also regulate cellular metabolism. Liver-specific deletion of BCL-2 interacting mediator of cell death (BIM) attenuates mitochondrial oxidative stress, hepatic steatosis, and ameliorates hepatic fatty acid metabolism in a mouse model of diet-induced obesity. Global BIM deletion increases mitochondrial respiration and lipid oxidation. Truncated phosphorylation-mediated inactivation of BH3 interacting-domain death agonist (BID) reduces carnitine palmitoyltransferase 1 (CPT-1), impairing fatty acid oxidation and promoting the accumulation of toxic lipid metabolites. A loss-of-function study showed that the proapoptotic BCL-2-associated death promoter (BAD) directly interacts with glucokinase (GK) and regulates hepatic energy metabolism by reducing fatty acid oxidation and gluconeogenesis while promoting glycolysis. Loss of hepatic BCL-2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3) results in increased mitochondrial mass, impaired gluconeogenesis, and reduced β-oxidation, and this phenotype is associated with elevated ROS production.

anaerobic ATP production through glycolysis and other hypoxia adaptative responses. The hypoxia also leads to reduced activity of the mitochondrial electron transport chain increasing ROS generation ($^{\cdot}O_2^-$ and subsequent H_2O_2). These oxidants modulate the activity of the PHD enzymes, which requires ferrous iron (Fe^{2+}) as a cofactor and its availability is reduced in the presence of excessive oxidants, inhibiting PHD activity and promoting HIF signaling [9]. The activation of HIF in response to hypoxia induces a series of adaptive changes that favor cell survival. Without these HIF-induced adaptations, hypoxia can suppress cell proliferation and leads to cell death. In a setting of cirrhosis, chronic liver injury induces fibrinogenesis, reducing vascularization, favoring hypoxia and HIF activation [56]. HIF plays an important role in the pathogenesis and pathophysiology of HCC, being a potential target for cancer therapy [56]. In a tumor environment, HIF activation contributes to the metabolic shift to the Warburg phenotype and promotes a mechanism to improve oxygen delivery, allowing the cell cycle progression, tumor development, and resistance to chemotherapeutic agents.

Taken together, ROS signaling can prevent or activate tumor formation. Physiological oxidative stress is a necessary biological response and excessive ROS production triggers oxidative stress, suggesting the pleiotropic nature of ROS signaling. Accordingly, inhibiting or reducing ROS levels may seem feasible strategies to cure liver disease, it is evident that ROS are also important for intracellular signals and cascades. In addition to classical ROS pathways, B-cell lymphoma 2 (BCL-2) proteins are emerging as physiological and pathophysiological associated redox molecules in cell survival and death.

BCL-2 PROTEINS: MODULATORS OF APOPTOSIS AND MITOCHONDRIAL OXIDATIVE STRESS IN LIVER DYSFUNCTION

Cellular ability to metabolize different energy-providing substrates (glucose, fatty acids, ketone bodies, and amino acids) is orchestrated by multiple complex biochemical pathways and cellular processes. Apart from serving as the source of ATP and ROS generation, mitochondria can act as an important mediator of the intrinsic pathway of apoptosis, a programmed cell death that is essential for maintaining cellular growth and development in organisms. The mitochondrial pathway of apoptosis is primarily regulated by the family of BCL-2 proteins, which include both anti- and proapoptotic proteins. They consist of four conserved BCL-2 homology domains (BH1–4). The BH3-only members (BIM, PUMA, NOXA, BID, BAD, BIK, and HRK/DP5) are considered death domain proteins in the family. These proteins interact with each other to initiate the apoptotic pathway by releasing proteins from mitochondrial intermembrane space to the cytosol [57]. Liver injury correlates with apoptosis activation and hepatocyte cell death in the transition from steatosis to HCC. Interestingly, antiapoptotic BCL-2 proteins are highly expressed in fatty livers [58–60]. Instead, hepatic apoptosis is associated with obesity- and inflammatory-mediated activation of proapoptotic BCL-2 members [58]. The break of balance in the ratio of pro-/antisurvival BCL-2 proteins favors a proapoptotic outcome, leading to mitochondrial membrane depolarization, cytochrome *c* release, and caspases activation [61]. For a comprehensive overview of apoptosis and cell death in HCC development, readers should refer to the following reviews [62, 63]. Interestingly, components of programmed cell death can also regulate cellular metabolism in multiple tissues indicating the existence of a more intricate relationship between cellular metabolism and apoptosis [64, 65] (Fig. 4).

Noncanonical and physiological role of BCL-2 proteins in liver

Recent studies suggest that in addition to the canonical function as regulators of apoptosis, BCL-2 proteins have been implicated in non-apoptotic functions including mitochondrial physiology, calcium homeostasis at the ER, unfolded protein response, substrate metabolism, and DNA damage response [57]. Interestingly, due to this noncanonical role in mitochondrial function, BCL-2 family proteins are also proposed to be key regulators of oxidative stress [66]. Indeed, we recently showed that liver-specific deletion of BIM, which is an activator BH3-only proapoptotic protein, attenuates mitochondrial oxidative stress, hepatic steatosis, and ameliorates hepatic fatty acid metabolism in a mouse model of diet-induced obesity [60]. In line with these findings, global BIM deletion was shown to increase mitochondrial respiration and lipid oxidation, reduce adiposity, and improve insulin sensitivity in vivo [67]. In permeabilized hepatocytes, tBID (another activator BH3-only protein) promotes the accumulation of toxic lipid metabolites by impairing fatty acid oxidation and reduces carnitine palmitoyltransferase 1 (CPT-1) activity [68]. Similarly, a loss-of-function study showed that the proapoptotic BAD directly interacts with glucokinase, an enzyme that catalyzed the first step of glucose metabolism to glucose-6-phosphate and deletion of BAD leads to β-cell dysfunction in mice [69]. Moreover, BAD regulates hepatic energy metabolism by reducing fatty acid oxidation and gluconeogenesis [65]. Enhanced mitochondrial fatty acid oxidation is known to increase electron flux to the electron transport chain resulting in ROS generation [70]. Therefore, considering the elevation in functional fatty acid oxidation in

BAD deficient hepatocytes, it is plausible that increased BAD activity may diminish ROS production and oxidative stress in the liver. In line with this, a loss of hepatic BNIP3, a proapoptotic BCL-2 family member, results in impaired hepatic glucose production coupled with reduced β-oxidation, and this phenotype is associated with elevated ROS production [71]. Since the loss of hepatic BNIP3 promotes increased mitochondrial mass, it is proposed that the overall impact of BNIP3 on hepatic metabolism is dependent on the proportion of functional mitochondria (in the total mitochondrial mass) capable of promoting hepatic fatty acid oxidation, and oxidative phosphorylation. These findings suggest that BIM, tBID, BAD, and BNIP3 have a differential effect on cellular ROS production in hepatocytes (Fig. 4). Thus, the role of BCL-2 family proteins is not limited to their apoptotic function but extends beyond their canonical apoptotic regulation and also includes other important elements of mitochondrial function and cellular energy metabolism. Detailed studies focused on understanding the molecular mechanisms controlling the switch from cell death to non-apoptotic role or vice versa are necessary to further explore the potential therapeutic options of these family members.

ROS-mediated regulation of BCL-2 proteins

BCL-2 proteins can contribute to hepatic ROS formation, in addition ROS levels can trigger cell death through BCL-2 protein modulation. The antiapoptotic BCL-2 protein is diminished in NAFLD/NASH and its overexpression can reduce hepatic apoptosis [72]. BCL-2 proteins regulate ROS/oxidative stress-mediated apoptosis, but can conversely be regulated by ROS signaling via phosphorylation and ubiquitination [73]. Hence, ROS can sensitize cells to apoptosis by suppressing cellular BCL-2 levels, which are critical to antiapoptotic activity. By detoxifying ROS, antioxidants may therefore reverse the ROS-induced decline in BCL-2 and prevent apoptosis. It is interesting to note that several studies consistently highlight that induction of oxidative stress is necessary for antitumor agents to efficiently target HCC, which contrasts with the ROS-mediated pathways involved in HCC development. Therefore, from a therapeutic standpoint, detailed studies investigating molecular pathways of ROS-mediated regulation of BCL-2 proteins (transcriptional, translational, or posttranslational modifications) will shed light on how the ROS-BCL-2 family crosstalk can be therapeutically exploited for HCC.

Targeting BCL-2 proteins for HCC treatment

BCL-2 family member proteins are an obvious pharmacological target for cancer. While the non-apoptotic role of BCL-2 proteins is relatively new, extensive research has been performed to increase our understanding of the mechanism of apoptosis regulation by these proteins. Indeed, this wealth of knowledge has led to several small molecules (antisense oligonucleotides and BH3-mimetic drugs) which have already been designed to target these proteins for cancer therapy, with several targets (BH3-mimetic drugs) entering the clinical phase [74]. Given the complex mechanism by which BCL-2 proteins orchestrate the apoptotic pathway, it is important to consider the interaction of member proteins with each other, their subcellular localization, and their varied mode of action, in order to develop a specific effect of distinct BCL-2 proteins on cellular metabolism. Novel nanotechnology approaches have been designed to improve the efficacy of the BCL-2 modulators in HCC (see below). Within the context of potential on-or off-target effects of member proteins on cellular metabolism, however, it is challenging to develop pharmacological modulators of BCL-2 proteins on energy homeostasis that is distinct from their pro or antiapoptotic function.

Breaking the balance in the expression and activity of BCL-2 family members can result in profound effects on metabolism and cell survival. This is of particular relevance for the development and clinical treatment for HCC and several other tumors. Indeed, the BCL-2-specific inhibitor venetoclax has shown efficacy against different cancers, with more than 290 registered clinical trials (clinicaltrials.gov). This positive outcome is built on many years of basic science focusing on BCL-2 proteins, which hopefully will result in substantial improvements for HCC patient care.

PROTEIN TYROSINE PHOSPHATASE (PTP) OXIDATION: A KEY MECHANISM DOWNSTREAM ROS PRODUCTION IN THE TRANSITION FROM STEATOSIS TO NASH AND HCC

PTPs and control of molecular signaling in hepatocytes

Protein tyrosine phosphatases (PTPs) are important modulators of the insulin receptor (IR), and their dysfunction plays a key role in insulin resistance in type 2 diabetes, a risk factor for HCC development (Fig. 5). When insulin binds to its receptor on the cell surface of tissues it leads to autophosphorylation of tyrosine residues of the IR by activating its exogenous kinase activity, which then further phosphorylates the tyrosine residues on the insulin receptor substrate (IRS) proteins. Opposite to protein tyrosine kinases (PTK), PTPs are a superfamily of enzymes that remove phosphate groups from phosphorylated tyrosine residues on proteins and inactivate the IR kinase activity, thereby ceasing the insulin signaling cascade. While several PTPs have been implicated in the regulation of insulin signaling, among those PTPN1 is critical in the regulation of the insulin pathway [60]. Both in vitro [75] and in vivo [76, 77] studies have demonstrated that PTPN1 inhibition is beneficial in alcoholic liver disease and acetaminophen-induced hepatotoxicity. In addition, PTPN2 with 72% overlapping identity to PTPN1 regulates insulin signaling and glucose homeostasis mainly in the liver [78]. Similarly, PTPN6, PTPN9, and PTPN11 are other PTPs among classical PTP family members that have been implicated in hepatic insulin signaling [79–82]. The balance between PTK and PTP signaling is essential for regulating various cellular pathways and perturbations to this balance lead to carcinogenesis [83]. Interestingly, the dysregulated activity of different PTPs has been implicated in HCC [84]. Most tyrosine phosphatases function as a tumor suppressor, but some PTPs can also be oncogenic depending upon the severity and progression of HCC [84].

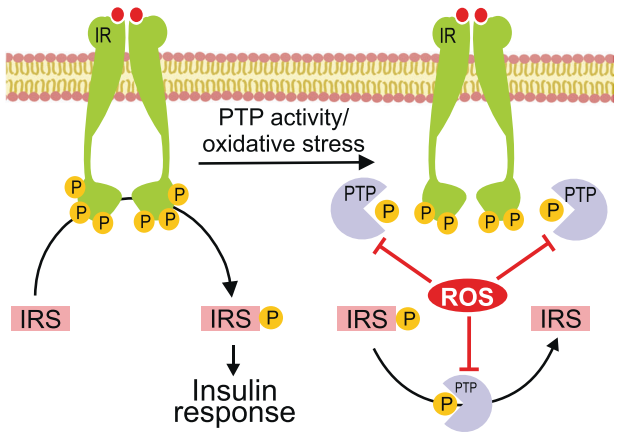

Fig. 5 Protein tyrosine phosphatases (PTPs) are important modulators of the insulin receptor (IR) and PTP oxidation plays a role in insulin resistance. When insulin binds to the IR it leads to autophosphorylation of tyrosine residues of the IR and activates its exogenous kinase activity, which then further phosphorylates the tyrosine residues on the insulin receptor substrate (IRS) proteins. PTPs remove phosphate groups from phosphorylated tyrosine residues on proteins and inactivate the receptor kinase thereby ceasing the insulin signaling cascade. Reactive oxygen species (ROS) can oxidize and inactivate the PTPs affecting physiologically and pathophysiological insulin signaling.

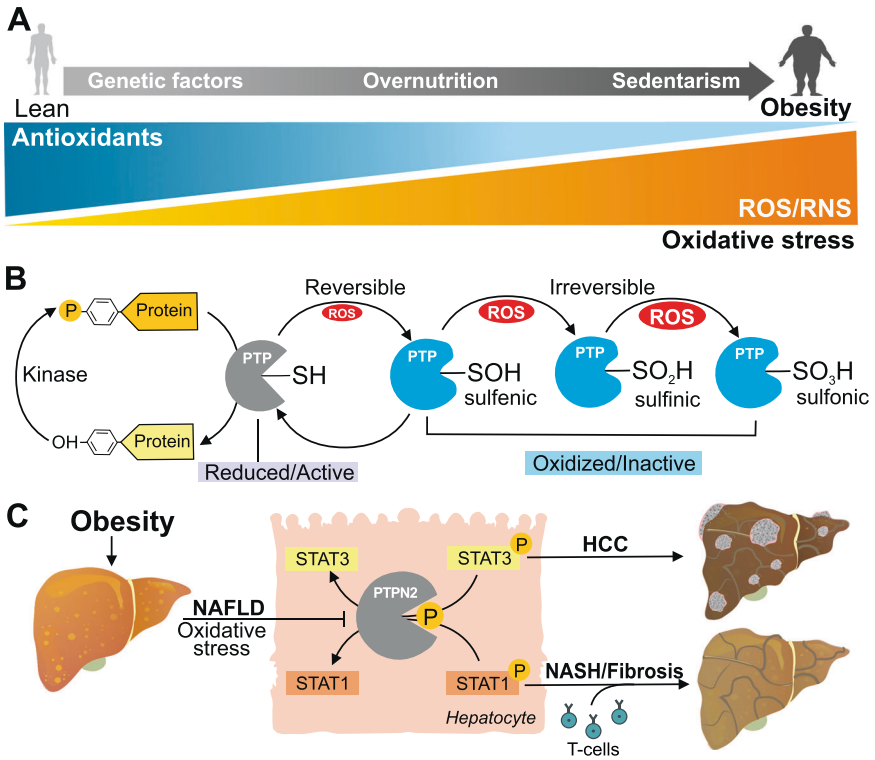

Fig. 6 Obesity contributes to the development of NASH and HCC by independent mechanisms. A Obesity is a multifactorial disease that manifests as consequential to environmental influences, mainly excessive consumption of calories (overnutrition) and a lack of physical activity (sedentarism), associated with genetic factors that predispose body fat accumulation. One of the consequences of obesity is an unbalance between the production of oxidant species and antioxidant capacity leading to oxidative stress generated by reactive oxygen species (ROS) and reactive nitrogen species (RNS). **B** Protein tyrosine phosphatases (PTPs) regulate tyrosine phosphorylation-dependent signal transduction through tyrosine dephosphorylation of protein substrates. The protein structure and presence of cysteine residue in the active site of PTPs with low pKa render members of this family of proteins highly susceptible to oxidation by ROS to the reversible form of sulfenic acid or irreversible forms of sulfinic and sulfonic acid accompanied by conformational changes that inhibit PTP activity and prevent substrate binding. **C** Obesity is frequently associated with nonalcoholic fatty liver disease (NAFLD), a condition that favors liver oxidative stress. Under this condition, STAT1 and STAT3 phosphatase protein tyrosine phosphatase non-receptor type 2 (PTPN2) is inactivated, thereby increasing STAT1 and STAT3 signaling. While heightened STAT1 signaling is responsible for the recruitment of activated cytotoxic T cells and ensuing NASH and fibrosis, this is not essential for HCC. Rather, STAT3 signaling promotes HCC without NASH and fibrosis.

Oxidative stress inactivates PTPs and promotes NASH and HCC in obesity

The levels of ROS formation and oxidative stress are significantly increased in obesity (Fig. 6A). PTPs are downstream targets of ROS and they have been shown to be regulated by reversible oxidation [85–87]. PTPs contain a conserved catalytic cysteine with an unusually low pKa, which promotes their active nucleophile function and makes them highly susceptible to inactivation by ROS (Fig. 6B) [88]. For example, PTPN1 undergoes both reversible and irreversible oxidation in HepG2 and A431 human cancer cells [89]. A study using cysteinyl-labeling assay demonstrated that platelet-derived growth factor (PDGF) induces reversible oxidation of classical tyrosine phosphatases PTPN11 and PTEN (tumor suppressor phosphatase) in angiomyolipoma cells [90]. Moreover, in a recent work PTP oxidation has been shown to be associated with the progression of gastric carcinoma [91]. Collectively these findings suggest the important role of PTP oxidation in cancer. It is reasonable to anticipate the role of oxidative inactivation of PTPs in hepatic cells. Indeed, we previously reported that ROS-mediated oxidative inactivation of PTPN2 leads to selective activation of an insulin-STAT5-IGF-1-GH pathway under insulin resistance, hence contributing to the progression of obesity in high-fat-fed mice [92]. In the same study, five receptor-type PTPs (PTPRA, PTPRC, PTPRE, PTPRK, and PTPRJ) were also oxidized but their role in hepatic signaling remains unknown. In addition, increased PTP oxidation has been demonstrated in human liver biopsies from NAFLD patients [93].

Accumulating evidence suggests that NASH increases the susceptibility to develop HCC by promoting hepatic fibrosis and cirrhosis [94]. However, the molecular mechanisms that give rise to HCC in some obese patients and not others remain largely unclear (Fig. 1). In a recent study, Grohmann et al. reported that obesity contributes to the development of NASH and HCC by independent mechanisms [93] (Fig. 6C). Building upon our earlier work showing obesity-induced oxidative inhibition of PTPN2 in the liver [92], it was found that that PTPN2 deficiency promoted NASH, fibrosis, and HCC in diet-induced obesity. To further delineate the mechanism, hepatocyte-specific PTPN2-deficient mice with heterozygous loss of either STAT1 or STAT3 were used. Interestingly, blocking STAT1 signaling prevented high-fat-fed mice from NASH/fibrosis but did not impact on HCC in the hepatocyte-specific PTPN2-deficient mouse model. On the contrary, attenuating STAT3 signaling did not affect NASH and fibrosis but rescued the HCC phenotype in hepatocyte-specific PTPN2-deficient mice fed a high-fat diet. These elegant experiments suggested that obesity-induced PTPN2 inactivation promoted STAT1 and STAT3 signaling, but each pathway had a differential impact on NASH/fibrosis and HCC development. Further studies in clinically relevant samples are required to clarify if the findings in mouse models are translatable to human HCC. These studies, collectively, have indicated that oxidative inhibition of PTPs serves as a mechanism by which optimal tyrosine phosphorylation is maintained under physiological conditions but, if dysregulated, can contribute to liver dysfunction and HCC development.

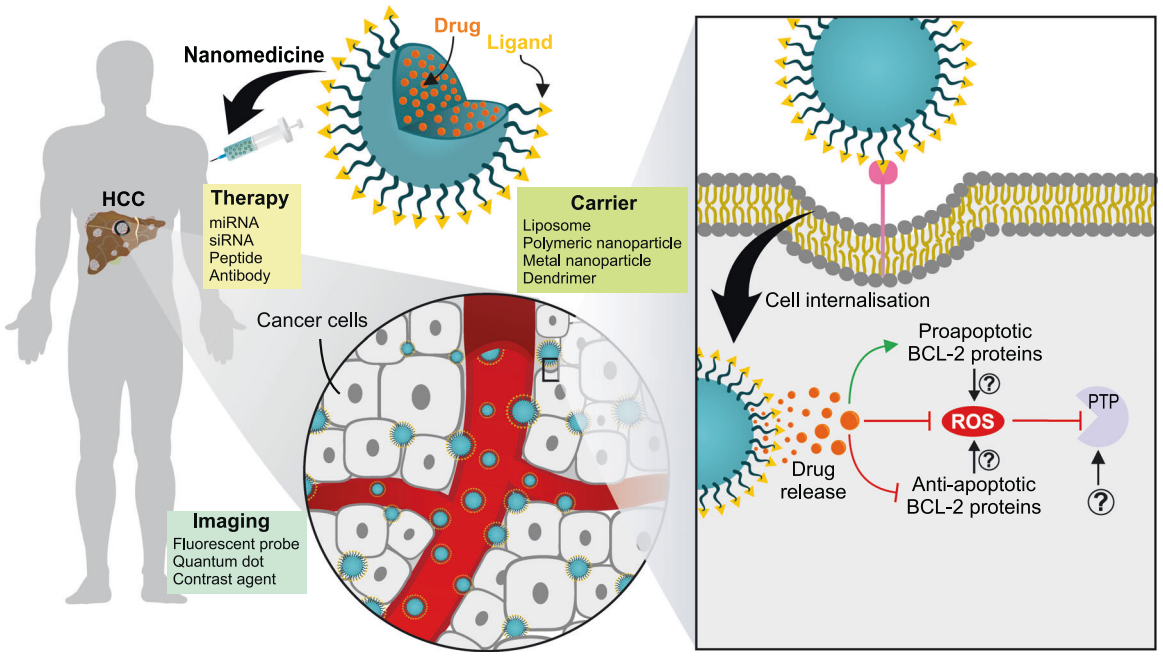

Fig. 7 Schematic illustration of nanotheronostic agents targeting oxidative stress-dependent pathways in obesity-associated HCC. Nanomedicine can be used for the delivery of therapeutic agents such as micro(mi)RNA, small interfering(si)RNA, peptides, or antibodies. The drug delivery approach needs to overcome a series of biological barriers to successfully target the molecular mechanisms of ROS production. The nanomedicine strategies should modulate BCL-2 proteins and either enhance ROS inhibitors or decrease ROS activators to prevent dysfunctional PTP activity.

The quest for PTP modulators

PTPN1 has emerged as a valid therapeutic target for the treatment of obesity and associated complications [88]. Moreover, antibodies [95] and small molecules [96] have been recently developed and are able to recognize and stabilize oxidized PTPN1. These experiments showed that insulin signaling could be improved by stabilizing the inactive form of oxidized PTPN1. With regard to oxidative inactivation of PTPs, this is an emerging field and detailed information on several aspects of the role of oxidized PTPs within the context of insulin resistance is lacking, including (1) identification of specific types of oxidized PTPs with significant contributions to impaired insulin signaling in liver dysfunction and HCC progression, (2) cell-specific contributions of oxidative inactivation of specific PTPs and their role in whole-body insulin resistance, and (3) in vivo impact of direct modulation of the oxidation status of PTPs without altering systemic metabolism.

Historically, tyrosine phosphatases were thought to have little specificity and therapeutic potential based on two observations: first, the catalytic subunits function nonspecifically to dephosphorylate many protein substrates; and second, a much lower number of genes encoding phosphatases relative to kinases. However, recent studies by several by several groups provide evidence that the PTP family exhibits similar complexity and specificity as PTKs. Given their known roles in HCC development, further understanding of mechanisms underlying the functions and regulation of PTPs by oxidative stress may have important applications for cancer diagnosis and therapy.

THERAPEUTIC CHALLENGES FOR NOVEL TECHNOLOGIES TARGETING OXIDATIVE STRESS-DEPENDENT PATHWAYS IN HCC

The standard treatment for early-stage liver tumors and for tumors at early stages not suitable for surgical therapies is radiofrequency ablation [97]. In intermediate-stage lesions, transarterial chemoembolization is established as the standard of care leading to median survivals of 2 years [97]. Nanomedicines, defined as intentionally designed therapeutics and diagnostics at the nanoscale (1–100 nm), offer a vast potential to overcome traditional chemotherapy limitations and have become a primary focus in the development of anticarcinogens. While small drug molecules often suffer from low aqueous solubility, poor bioavailability, and permeability [98], nanoparticles can serve as nanoscale drug vehicles by physically or chemically encapsulating the drug (Fig. 7). The surface of nanoparticles can be decorated with targeting ligands to allow drug delivery with enhanced selective accumulation and reduced nonspecific cytotoxicity [99]. In addition, surface modifications alter the physicochemical properties of the nano-entities, thereby shielding them from degradation and undesired cellular uptake, prolonging their blood circulation, and allowing their penetration through various biological barriers.

A variety of nanomaterials, inclusive of liposomes, silica nanoparticles, micelles, and polymeric nanoparticles, have been explored for targeting oxidative stress in HCC, primarily on the inhibition of antiapoptotic BCL-2 proteins (Table 1). The passive targeting strategies utilize defective vascular structure and impaired lymphatic drainage in HCC to achieve targeted nanoparticle drug delivery via the known enhanced permeation and retention (EPR) effect. However, the EPR effect provides only a modest specificity towards the target tumor and is highly dependent on the physicochemical characteristics of the nanoparticle and the intrinsic tumor biology. In comparison, active targeting can significantly improve the local drug concentration delivered through modifications of nanoparticles with affinity ligands to bind with receptors expressed on tumor cells. Studies have demonstrated target HCC therapies through different cell surface receptors (i.e., asialoglycoprotein receptor (ASGPR) [100], folate receptor [101], or CD44 [102]). Nevertheless, the majority of these receptors are common tumor biomarkers that are not HCC specific and cannot be used as predictive markers to monitor the therapeutic efficacy of nanomedicines. Signaling proteins of oxidative stress-dependent pathways, such as BCL-2 and PTPN1, could be ideal for therapeutic prediction, but their intracellular

Table 1. Summary of nanomedicine studies for the treatment of HCC.

Ligand	Receptor	Drug	Target	Nanocarrier	Ref.
Passive Targeting		NuBCP-9	BCL-2	PEG- polypropylene glycol-PEG-modified polylactic acid	[109]
		Betulinic acid	BCL-2	Poly(lactic-co-glycolic acid)	[110]
		Arsenic trioxide	SH2-PTP1	ZnAs/ silica	[111]
		Linolenic acid(/tuftsin)	BCL-2	Liposomes	[112]
		Ursolic acid	BCL-2	Poly(N-vinylpyrrolidone)-block-poly (ε-caprolactone)	[113]
		siRNA(/epirubicin)	BCL-2	Liposomes	[114]
		Bcl-2 conversionNur77 gene (/paclitaxel)	BCL-2	Poly[(R)-3-hydroxybutyrate] (PHB)-b-poly(2-(dimethylamino)ethyl methacrylate)	[115]
Hyaluronic acid	CD44	ABT-199(/doxorubicin)	BCL-2	Cationic amphipathic starch	[102]
Glycyrrhetinic acid(GA) Hyaluronic acid	GA receptor CD44	siRNA(/Doxorubicin)	BCL-2	1,2-distearoyl-sn-glycero-3-phosphoethanolamine-polyethylene glycol-polyetherimide	[116]
Cathepsin B-specific cleavable peptide	Cathepsin B	Navitoclax(/doxorubicin)	BCL-2	Cathepsin B-specific cleavable peptide	[117]
Lactoferrin	ASGPR	Imatinib mesylate	BCL-2	PEGylated liquid crystalline	[100]
Galactose	ASGPR	miRNA-122(/5-fluorouracil)	BCL-2	Chitosan	[118]
Folic acid	Folate receptor	siRNA(/doxorubicin)	BCL-2	Poly(ε-caprolactone) /linear poly (ethylene imine)	[101]
Folic acid	Folate receptor	ABT-737(/diacid metabolite of norcantharidin)	BCL-2	Lipid bilayer/mesoporous silica	[119]
Galactose	ASGPR	siRNA (/doxorubicin)	BCL-2	poly[2-(dimethylamino)ethyl methacrylate]/ poly(3-azido-2-hydroxypropyl methacrylate)	[104]

expression renders them unsuitable for clinical imaging. The discovery of new markers and ligands for tumor targeting oxidative stress inhibition and therapeutic prediction in HCC still represents a major challenge in the development of novel nano-drug systems and combination therapies.

Recent advances in nanomedicine for more efficient personalized treatment with minimized side effects have generated nanotheranostic systems via an "all-in-one" approach. The confluence of therapeutics and diagnostics enables disease monitoring, tissue imaging, and therapeutic efficacy evaluation simultaneously or sequentially [103] (Fig. 7). A representative study of an integrated nanotheranostic system achieved enhanced treatment of HCC via targeting oxidative stress, showing a unique capability in real-time imaging for drug tracing and pH stimuli-responsive drug release [104]. However, whereas a large number of these advanced therapeutics have demonstrated a promising preclinical efficacy, none of them have advanced past clinical trials. The limited success could be mainly attributed to the challenges presented by tumor heterogeneity, rapid blood clearance, and discrepancies between animal models and human tumors. Another major obstacle that may have been overlooked is the pathophysiologic barrier caused by aberrant tumor interstitium in obesity-associated HCC in vivo. The increased interstitial fluid pressure and deformed extracellular matrix in HCC can significantly affect the delivery efficiency during the interstitium-to-cell-membrane transportation of nanoparticles. Such effect could be further exacerbated in liver cancer, as an elevated interstitial fluid pressure was observed in deteriorating chronic liver disease with an increased ROS level [105].

In addition, accumulating evidence has suggested that the physicochemical properties of devised nanoparticles can be readily altered by plasma proteins in the blood, forming a so-called "protein corona" via protein–nanoparticle interactions. The adsorbed proteins partially or completely cover the engineered nanoparticles' surface, giving them new identities (i.e., size, shape, and surface charge) that determine the stability, biodistribution, toxicity, pharmacokinetics, and the ultimate physiological response [106]. As such, controlling the protein adsorption by modifying nanoparticles with "stealth" functional ligands [107] or tailoring nanoparticles which favor selective endogenous peptides [108] would bring therapeutic benefits for the design of effective yet safe therapeutic technologies for targeting oxidative stress in HCC.

PERSPECTIVES

It is now clear that oxidative stress is an important contributor to disease progression in NAFLD-associated HCC. In this review, we have summarized the main sources of hepatic ROS formation, signaling pathways affected as well as therapeutic challenges concerning oxidative stress in the pathophysiology and treatment of HCC. ROS plays an important regulatory role in physiological cellular processes, including inflammation and insulin signaling. However, excessive accumulated ROS in metabolic tissues in obesity is detrimental to cellular function. Understanding the molecular mechanisms behind ROS production in physiology and pathophysiology is necessary for the design of novel therapies in HCC. Thus, the critical pharmacology quest in the field is restoring normal levels, source, and timing of ROS production aiming to reduce oxidative stress and improve cell signaling and function. Funding agencies and foundations should prioritize multidisciplinary international consortiums with long-term projects in HCC, aiming to target basic biochemical science, metabolism, medicine, and nanotechnology. We anticipate that collaborative studies in the future between scientists and clinicians will provide innovative diagnostic and therapeutic strategies for obesity-associated HCC.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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