Cellular & Molecular Biology Letters

REVIEW Open Access

The spatiotemporal heterogeneity of reactive oxygen species in the malignant transformation of viral hepatitis to hepatocellular carcinoma: a new insight

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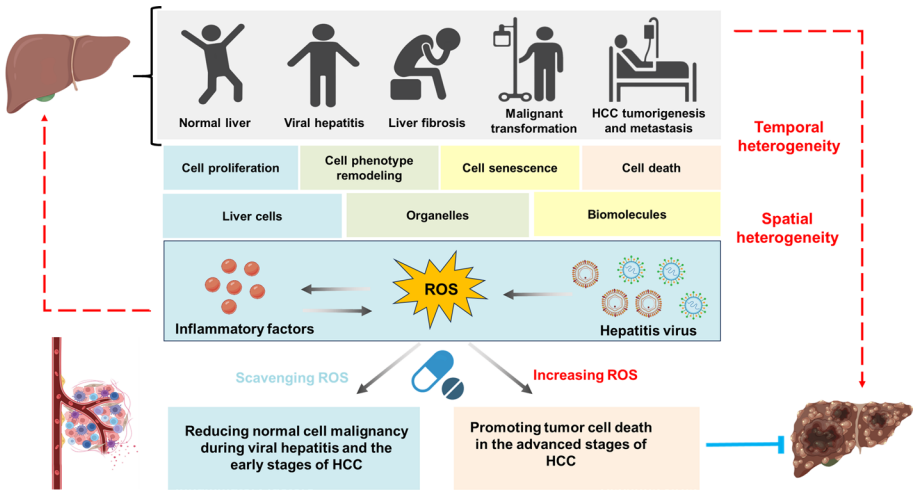
Abstract

During the transformation of viral hepatitis into hepatocellular carcinoma (HCC), oxidative stress levels increase significantly, leading to tissue damage and chronic inflammation. HCC is characterized by spatiotemporal heterogeneity, which influences oxidative stress patterns, with reactive oxygen species (ROS) as the primary representative molecules. ROS serve not only as critical biomarkers of cancer but also as potential therapeutic targets for HCC, given that their increased levels can either promote or inhibit disease progression. In this review, we systematically examine the temporal heterogeneity of ROS, emphasizing its role in different stages of HCC progression caused by viral hepatitis and in influencing cell fate. We further explore ROS spatial heterogeneity at three levels: cellular, organelle, and biomolecular. Next, we comprehensively review clinical applications and potential therapies designed to selectively modulate ROS on the basis of its spatiotemporal heterogeneity. Finally, we discuss potential future applications of novel therapies that target ROS spatiotemporal heterogeneity to prevent and manage HCC onset and progression. In conclusion, this review enhances understanding of ROS in the progression of viral hepatitis to HCC and offers insights into developing new therapeutic targets and strategies centered on ROS heterogeneity.

Keywords: Reactive oxygen species, Spatiotemporal heterogeneity, Viral hepatitis, Hepatocellular carcinoma, Treatment strategy

Graphical Abstract



Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer-related mortality, with chronic liver inflammation, particularly hepatitis B or C infection, accounting for approximately 80–90% of HCC cases [1, 2]. Since the early 2000s, oxidative stress levels have been found to have significantly increased in patients with viral hepatitis and HCC, as indicated by elevated levels of malondialdehyde (MDA), 4-hydroxynonenal (HNE), and advanced oxidation protein products (AOPP) in liver tissue and blood, along with a decrease in antioxidant enzyme activity, particularly superoxide dismutase (SOD) [3–6]. HCC is a complex disease with significant heterogeneity across its progression, encompassing morphological, epigenetic, transcriptomic, proteomic, post-translational modification (PTM), metabolomic, and tumor microenvironment (TME) variations [7, 8]. HCC heterogeneity influences oxidative stress variability during disease progression, shown by fluctuating reactive oxygen species (ROS) levels. Specifically, ROS heterogeneity manifests in different effects across disease stages and cellular fates (temporal heterogeneity) and in varied impacts on liver cells, organelles, and molecules (spatial heterogeneity).

ROS play crucial roles as second messengers in numerous intracellular signaling cascades at normal physiological levels [9]. However, at high levels, ROS can cause indiscriminate damage to biomolecules, resulting in functional loss and even cell death [10]. Oxidation of nucleic acids, lipids, and proteins is a key factor in tissue damage caused by oxidative stress, which alters their structure and function, produces toxic intermediates, and leads to various negative effects within cells [11, 12]. While ROS have been identified as contributing factors in the development of HCC induced by chronic hepatitis, a comprehensive understanding of their role in the malignant transformation of chronic hepatitis remains incomplete, particularly regarding their time- and spatial-dependent implications. Accordingly, a more profound comprehension of ROS heterogeneity

is instrumental in delineating its divergent roles within varying temporal and spatial contexts.

This review comprehensively explored the spatiotemporal heterogeneity of ROS in the progression of viral hepatitis-induced HCC. We scrutinize the role of ROS from hepatitis onset through liver fibrosis to HCC growth and migration, discussing their impact on cellular fates such as proliferation, phenotype remodeling, senescence, and cell death. Moreover, we explore the effects of ROS at different cellular, organelle, and biomolecular levels, while providing an overview of clinical applications and potential therapeutic agents for selectively modulating ROS on the basis of spatiotemporal heterogeneity. This review thus offers a theoretical framework for understanding ROS heterogeneity during the progression from viral hepatitis to HCC, providing insights for potential therapeutic interventions.

Comparison of ROS between traditional perspectives and the murburn model

ROS are oxidatively active molecules or ions that contain unpaired electrons. They primarily include superoxide anion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and singlet oxygen ($^1O_2$) [13]. In classical biochemistry, ROS are viewed largely as harmful byproducts generated during cellular metabolism. Their formation is most prominent in mitochondria, where components of the electron transport chain, specifically complex I and complex III, occasionally leak electrons that react with molecular oxygen to produce superoxide anion. This electron leakage becomes especially significant under conditions of high mitochondrial membrane potential or excess substrate supply [14]. In addition to the mitochondrial sources, the NOX enzyme system (located on the plasma membrane, endoplasmic reticulum, and endocytosome membrane) catalyzes electron transfer from NADPH to O_2 to produce $\cdot O_2^-$. This process is critical for immune cells, such as neutrophils and macrophages, during the respiratory burst used to clear pathogens [15]. Other enzymes, including xanthine oxidase (XO) and lipoxygenase (LOX), contribute to ROS generation during the metabolism of various substrates [16].

To counterbalance these effects, cells deploy a robust antioxidant defense system. Key components include superoxide dismutases (SODs, such as the cytosolic SOD1, mitochondrial SOD2, and extracellular SOD3) that efficiently dismutate $\cdot O_2^-$ into H_2O_2 and O_2 [17]. Catalase (CAT), primarily located in the outer mitochondrial membrane and peroxisomes, decomposes H_2O_2, while glutathione peroxidase (GPX) reduces H_2O_2 and organic peroxides using reduced glutathione (GSH), generating water and corresponding alcohols and oxidizing GSH to glutathione oxidized (GSSG) [18, 19]. In addition, small-molecule antioxidants such as vitamin C, vitamin E, coenzyme Q10, and GSH participate in maintaining cellular redox balance by scavenging free radicals and interrupting chain reactions [20].

In contrast to the classical view, the murburn model (a contraction of "mured burning") redefines the role of ROS, referred to in this context as diffusible ROS (dROS), as essential catalytic intermediates rather than damaging byproducts [21]. According to this model, dROS such as superoxide anion and H_2O_2 are not merely metabolic waste; they actively mediate reactions outside the confines of the enzyme active site through free radical mechanisms [22].

In the murburn framework, dROS directly facilitate ATP generation by interacting with ADP and phosphate. This process bypasses the need for transmembrane proton gradients or the rotational catalysis of ATP synthase (complex V). Instead, energy conversion and metabolic regulation are maintained by a network of dynamic reactions at the membrane interface and in the mitochondrial matrix [23]. Redox centers in complexes I, II, III, and IV (including flavin, heme, Fe–S clusters, and Cu centers) participate in a distributed generation of dROS. These complexes transfer electrons to molecular oxygen, forming a variety of reactive species that are buffered by coenzyme Q and cytochrome c [24]. This distributed, parallel mode of dROS generation allows multiple one-electron active centers to jointly ignite the mitochondrial membrane interface, thereby promoting ATP synthesis without necessitating a strictly ordered electron transport chain.

Moreover, the murburn model emphasizes that the dROS generated in the unique environment of the mitochondrial membrane, marked by limited proton availability and specific dielectric properties, can persist for longer durations near the membrane. This sustained presence forms a localized "murzone" where dROS effectively couple ADP and phosphate to produce ATP [25]. Should these reactive species diffuse into the bulk aqueous phase, conventional antioxidant enzymes (SOD, CAT, and GPX) act quickly to neutralize them, thereby preventing uncontrolled oxidative damage. Despite its explanatory potential, the murburn model remains a subject of vigorous debate, necessitating rigorous experimental validation for its proposed catalytic paradigm.

The ROS-regulated signal pathways in the progression from viral hepatitis to HCC

The cellular response to ROS is tightly regulated through multiple signal pathways, among which the ROS-regulated signal pathways are of particular significance. These pathways not only mediate the cellular defense mechanisms against oxidative damage but also significantly influence the dynamic changes that occur during the transition from viral hepatitis to HCC. This section will delve into three key ROS-regulated signal pathways: the nuclear factor erythroid 2-related factor (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1) pathway, the nuclear factor kappa-B (NF-κB) pathway, and the mitogen-activated protein kinase (MAPK) pathway, to elucidate their individual roles and interconnections in the context of the progression from viral hepatitis to HCC.

Nrf2/Keap1

The Nrf2/Keap1 axis constitutes a master redox sensing pathway that governs the hepatic response to oxidative stress. Under homeostatic conditions, Nrf2 is sequestered in the cytoplasm by its adaptor protein Keap1 and targeted for proteasomal degradation. Modest increases in ROS modify critical cysteine residues on Keap1, impairing its ubiquitin ligase activity and allowing newly synthesized Nrf2 to escape degradation [26]. Stabilized Nrf2 subsequently translocates into the nucleus, where it binds antioxidant response elements (AREs) in the promoters of target genes heme oxygenase-1 (HO-1), SOD1, CAT, NADPH quinone oxidoreductase 1 (NQO1), and glutathione *S*-transferase (GST), thereby mounting a robust antioxidant and cytoprotective program [27, 28]. Autophagy adaptor p62 can further amplify this response by competing with Nrf2 for

Keap1 binding, while thioredoxin reductase-1 (TrxR1) fine-tunes Nrf2 activity through redox-sensitive modifications [28, 29].

Numerous preclinical studies have confirmed that Nrf2 activation protects against hepatocyte apoptosis, inflammation, and fibrosis, delaying the onset of cirrhosis and early tumorigenesis [30, 31]. However, mounting evidence also implicates sustained Nrf2 hyperactivation in the later stages of liver disease. In human and mouse models of HCC, elevated Nrf2 activity correlates with enhanced tumor cell proliferation, and metabolic reprogramming [32, 33]. Together, these findings underscore the dual role of the ROS-Nrf2/Keap1 pathway in liver pathology. On the one hand, transient Nrf2 activation is indispensable for detoxifying ROS and preserving hepatocyte integrity during acute injury. On the other hand, chronic Nrf2 overactivation can be coopted by malignant hepatocytes to support growth and survival in the oxidative tumor microenvironment.

NF-κB

ROS exert a central influence on NF-κB signaling, orchestrating both inflammatory and adaptive responses that drive liver disease progression. In resting cells, NF-κB is retained in the cytoplasm by its inhibitor IκB. Upon stimulation by inflammatory cytokines, bacterial components or ROS, IκB is phosphorylated by the IκB kinase complex and subsequently degraded [34]. Freed NF-κB then translocates to the nucleus, where it initiates transcription of a broad gene program. This includes pro-inflammatory cytokines (*IL-1β* and *TNF-α*), chemokines (*CCL2*), fibrogenic mediators (*TGF-β* and connective tissue growth factor), and antioxidant enzymes (*SODs*, *GPX*, *TrxR1*, *NQO1*, and *HO-1*) [35]. ROS themselves modulate NF-κB activation in a concentration-dependent manner. Moderate ROS levels enhance upstream kinase cascades that activate IκB kinase (IKK), whereas excessive ROS can oxidize critical thiol groups, such as Cys62 in the p50 subunit, impairing NF-κB to bind DNA [36]. Antioxidant molecules including *N*-acetylcysteine, alpha-lipoic acid, metallothioneins, and pyrrolidine dithiocarbamate, as well as overexpression of catalase or glutathione peroxidase, blunt ROS-driven NF-κB activation and underscore its role as a redox-sensitive transcription factor [20].

In the liver, this dynamic feedback loop produces opposing outcomes. Transient NF-κB activation promotes hepatocyte survival, regeneration, and antioxidant defenses, mitigating acute injury. In contrast, chronic NF-κB signaling sustains inflammation, drives hepatic stellate cell activation and extracellular matrix deposition, and fosters cirrhosis and HCC [37]. Conversely, loss of NF-κB function triggers unchecked hepatocyte apoptosis, spontaneous liver injury, and fibrosis [38]. Thus, ROS-regulated NF-κB signaling sits at the nexus of viral hepatitis, fibrogenesis, and HCC development, influencing both the initiation and progression of liver disease.

MAPKs

ROS serve as both upstream activators and downstream effectors of the MAPK pathway, playing a pivotal role in liver disease progression. Upon viral hepatitis, elevated ROS levels rapidly trigger phosphorylation of MAPK family members, including extracellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNK), and p38 MAPKs.

This phosphorylation cascade propagates signals that govern hepatocyte proliferation, survival, apoptosis, and inflammatory mediator production [39].

Among these kinases, ERK1/2 activation generally confers cytoprotective effects by upregulating antioxidant defenses and promoting hepatocyte regeneration [40]. In contrast, JNK and p38 signaling are closely linked to hepatocyte apoptosis, stellate cell activation, and fibrogenesis [41]. ROS-induced JNK activation increases expression of the pro-apoptotic protein Bim, which amplifies mitochondrial dysfunction and lipid peroxidation. Bim-deficient mice display reduced oxidative injury, preserved mitochondrial function, and attenuated fibrosis [42]. Moreover, sustained JNK-1 activation drives secretion of TNF-α and IL-6, perpetuating chronic inflammation and creating a microenvironment conducive to HCC development [43].

To prevent runaway MAPK signaling, hepatocytes induce expression of dual-specificity phosphatases, most notably MKP-1, which dephosphorylate and inactivate MAPKs, forming a negative feedback loop that tempers ROS-driven kinase activity [44]. However, chronic oxidative stress can overwhelm this brake, resulting in persistent MAPK activation, ongoing inflammation, and progressive fibrogenesis. Notably, MAPKs can also influence cellular redox status: activated ERK enhances antioxidant gene expression, while JNK further exacerbates ROS production [39]. In brief, ROS plays a crucial role in the chronic inflammation, fibrosis, and apoptosis caused by viral hepatitis by regulating the ERK, JNK, and p38 MAPK signaling pathways, thereby driving the occurrence and progression of HCC.

The temporal heterogeneity of ROS in the malignant transformation of viral hepatitis to HCC

The temporal variability of ROS is evident in its diverse effects at different stages of viral hepatitis-related HCC progression and on cell fate [12]. Chronic hepatitis caused by viral infection undergoes uncontrolled inflammation and liver fibrosis, ultimately leading to the onset and development of HCC. During disease progression and treatment, ROS activate different cellular functions depending on their concentration, distribution, and specific cell types. At low concentrations, H_2O_2 generates minimal ROS, which facilitate cell proliferation, differentiation, migration, and angiogenesis. At elevated H_2O_2 concentrations, ROS production increases, triggering inflammatory cascades that promote liver fibrosis, tumorigenesis, and metastasis. When cellular ROS levels exceed the tolerance threshold, they induce cell death and inhibit growth, a process known as "oxidative distress" signaling (Fig. 1) [45, 46]. The following sections will primarily explore the regulatory effects of ROS on various disease stages and cell fates.

HCC development

Viral hepatitis

ROS play a significant role in promoting chronic inflammation during persistent hepatic viral infections, which causes the development of liver fibrosis [47]. As early as 1983, empirical evidence demonstrated that hepatitis B virus (HBV) infection elicited oxidative stress in infected cells, ultimately culminating in cell death [48]. Subsequently, in 1996, researchers unveiled the occurrence of oxidative stress during the course of chronic-hepatitis-induced hepatitis C virus (HCV) infection and its close correlation

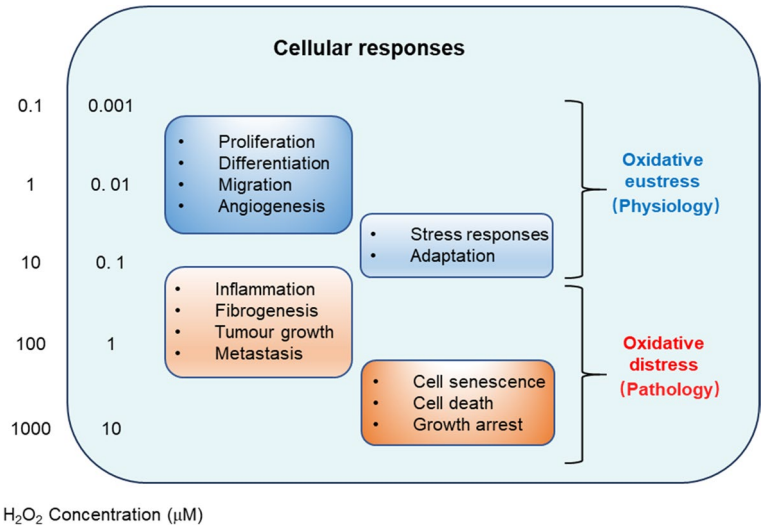


Fig. 1 Different concentrations of H_2O_2 with regard to cellular responses: oxidative eustress and oxidative distress. The effect of ROS on cells is indirectly reflected by the effect of H_2O_2 on cells. Low levels of H_2O_2 and its associated physiological redox signals are known as "oxidative stress." The specific manifestations are cell proliferation, differentiation, migration, and angiogenesis. However, elevated concentrations of H_2O_2 can trigger an inflammatory cascade that eventually induces growth arrest and cell death, known as "oxidative distress" [45, 46]. H_2O_2, hydrogen peroxide; ROS: reactive oxygen species. (Image created with BioRender.com, with permission)

with liver injury [6, 49]. HBV can integrate into the host genome, where expressed viral proteins induce chronic inflammation and promote HCC development [50]. Hepatitis B virus X protein (HBx), a vital protein in HBV pathogenesis and viral transcription, initiates oxidative stress by activating mitochondrion calcium-dependent signaling pathways, which in turn activate Nrf2, NF-κB, and MAPK pathways [50–52].

In contrast to HBV, HCV does not possess direct oncogenic properties and lacks the capacity for integration into the host genome, contributing to HCC pathogenesis primarily through chronic inflammation [53]. HCV proteins such as nonstructural protein 3 (NS3), nonstructural protein 5A (NS5A), envelope protein 1 (E1), envelope protein 2 (E2), and nonstructural protein 4B (NS4B) are closely interrelated with oxidative stress, activating a series of signaling pathways [54–57]. The molecular cascade associated with ROS subsequently triggers the increased expression of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-1β, and IL-18, fostering a vicious cycle of ROS and an inflammatory microenvironment within the liver (Fig. 2) [58, 59]. In addition, the endoplasmic reticulum, lysosomes, and liver microsomes in viral hepatitis also produce ROS [60, 61].

Also, chronic hepatitis triggers a "respiratory burst" characterized by increased oxygen absorption, which leads to the release and accumulation of ROS at the injury site. This creates a relentless cycle of inflammation and oxidative stress, perpetuating a hostile microenvironment that damages normal epithelial and stromal cells over time. The sustained inflammatory and oxidative environment poses a long-term risk for HCC development [62]. The activation of diverse inflammatory factors and signaling pathways, triggered by oxidative stress, is pivotal in the intricate pathogenesis of HCC. One such mediator is TNF-α, a pro-inflammatory agent produced by Kupffer cells and other

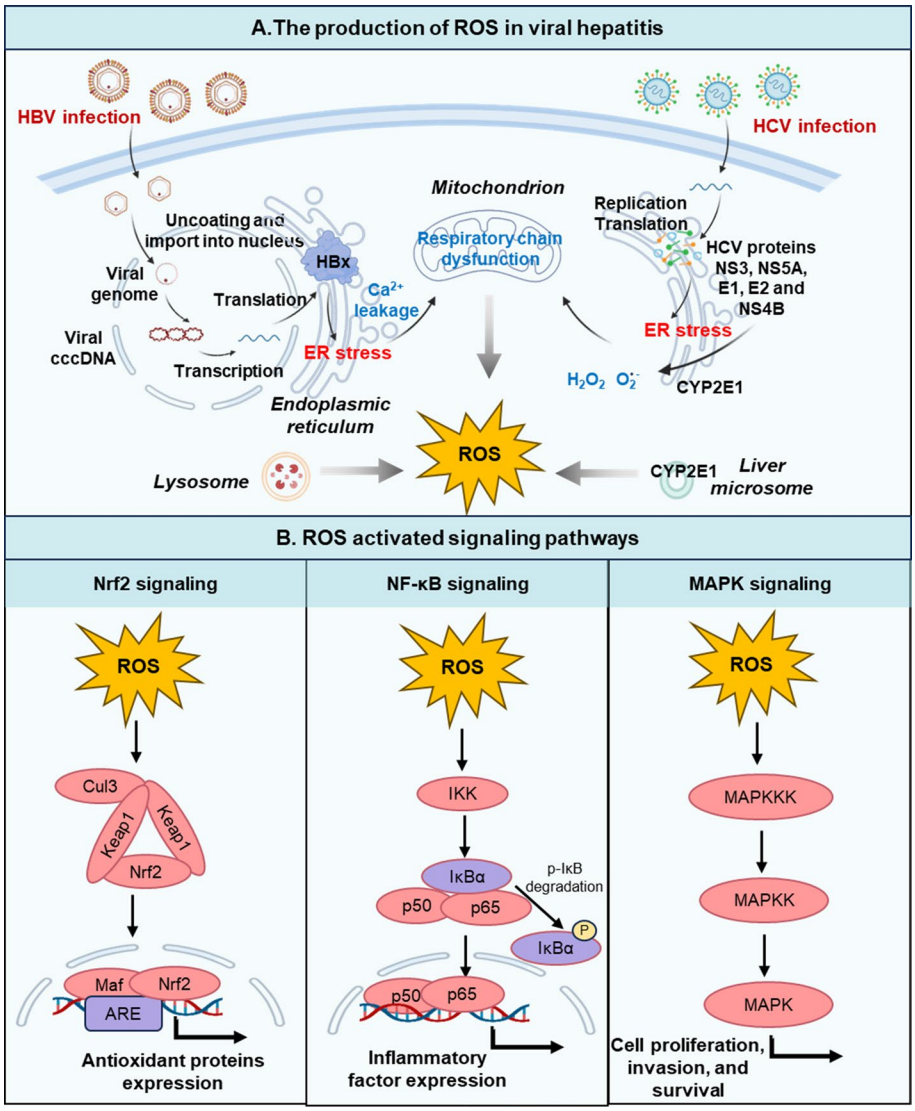


Fig. 2 The process of ROS generation and subsequent activation of signaling pathways in various forms of viral hepatitis. **A** In HBV and HCV hepatitis, viral protein accumulation in the ER following transcription and translation promotes ER stress and increases Ca^{2+} leakage. The leakage disrupts the mitochondrial electron transport chain (ETC), leading to increased ROS production. Additionally, in HCV hepatitis, NS5A induces CYP2E1, further enhancing ROS generation. Moreover, lysosomes and peroxisomes are significant organelles for ROS production. **B** Virus-induced ROS activate Nrf2, NF-κB, and MAPK signaling pathways. Cellular ROS production triggers corresponding responses, enhancing Nrf2 nuclear translocation and transcription of antioxidant genes to augment antioxidant capacity, thereby clearing ROS and preventing cellular damage from ROS accumulation. Furthermore, ROS activate the IKK pathway to activate NF-κB signaling, promoting nuclear translocation of p65 and p50 subunits and enhancing transcription and secretion of pro-inflammatory cytokines. Additionally, ROS activate the MAPK pathway, regulating cell proliferation, migration, and survival. ROS, reactive oxygen species; HBV, hepatitis B virus; HCV, hepatitis C virus; ER, endoplasmic reticulum; ETC, electron transport chain; NS5A, nonstructural protein 5A; CYP2E1, cytochrome P450 family 2 subfamily E member 1; Nrf2, nuclear factor erythroid 2-related factor; NF-κB, nuclear factor kappa-B; MAPK, mitogen-activated protein kinase; IKK, IκB kinase; p65, nuclear factor-κB subunit p65; p50, nuclear factor-κB subunit p50. (Image created with BioRender.com, with permission)

immune cells in response to tissue damage. Under the influence of NF-κB signaling, TNF-α propels the production of a repertoire of cytokines, orchestrating a cascade that recruits inflammatory cells, incites respiratory bursts, and escalates ROS levels [59, 63].

Furthermore, ROS-induced IL-6 contributes to HCC progression by suppressing IL-12 expression and programmed cell death 4 (PDCD4)-mediated apoptosis [64, 65]. Oxidative stress-induced inflammation further collaborates with the expression of oncogenes, exemplified by *cellular-myelocytomatosis viral oncogene (c-Myc)* and *Kirsten rat sarcoma viral oncogene homolog (k-Ras)*, promoting cell proliferation and accumulating oxidative DNA damage, thus accelerating the development of HCC [66, 67]. Consequently, in cases of viral hepatitis, excessive ROS generation triggers sustained hepatic inflammation and immune-driven oxidative stress, fostering HCC development.

Liver fibrosis

The vicious cycle of ROS and chronic hepatitis promotes the occurrence of liver fibrosis. As early as 1989, Mario Chojkier and colleagues proposed that oxidative stress directly contributes to the pathogenesis of liver fibrosis [4]. Specific lipid peroxidation markers, such as F2-isoprostane, HNE, and MDA, which are significantly elevated in liver fibrosis, directly trigger hepatic stellate cells (HSCs) activation and collagen gene expression [68]. Further, owing to impaired mitochondrial autophagy, leakage of mtDNA and mitochondrial damage-associated molecular patterns (mDAMPs) in the cytoplasm activate an autocrine inflammatory response, which induces inflammatory response in neighboring hepatocytes and Kupffer cells [69]. In this case, ROS-mediated mDAMPs play an essential role in promoting the proliferation of HSCs and their differentiation into collagen-secreting myofibroblasts, contributing significantly to the development of fibrosis [70].

Additionally, ROS facilitates the progression of hepatitis to cirrhosis. The accumulation of ROS in cirrhotic patients decreases intestinal permeability, allowing bacteria and bacterial products (e.g., endotoxin) to translocate from the intestinal lumen into the systemic circulation, which in turn causes further complications, including HCC [71]. Bacterial DNA is detectable in the blood and ascites of 30–40% of patients with liver cirrhosis, and plasma levels of LPS-binding protein (LBP) and IL-6 are significantly elevated [72]. In summary, the malignant cycle of oxidative stress and chronic inflammation contribute to the progression of liver fibrosis and subsequent tissue cirrhosis, which in turn elevates the risk of HCC.

Accumulation of mutations and malignant transformation

ROS play a critical role in the progression from viral hepatitis to HCC by driving the accumulation and malignant transformation of mutated genes. Excessive ROS oxidizes DNA bases, notably converting guanine into 8-hydroxy-2′-deoxyguanosine (8-OHdG) [73]. Elevated levels of 8-oxo-dG in liver tissues from chronic hepatitis patients and in HBx-transgenic mouse models correlate with precancerous lesions and HCC occurrence [74]. Moreover, HBV or HCV infection has demonstrated that infected cells produce higher ROS levels and suffer increased DNA damage compared with uninfected controls [75, 76]. In addition to base modifications, ROS induces single- and double-strand DNA breaks. Although single-strand breaks are typically repaired via the base excision repair (BER) pathway, persistent oxidative stress overwhelms repair capacity, resulting in double-strand breaks. These severe lesions can precipitate chromosomal rearrangements, deletions, or insertions via nonhomologous end joining (NHEJ) [77]. Chronic oxidative

stress also impairs DNA repair mechanisms by damaging repair proteins, thus reducing repair efficiency and allowing mutations to persist. In the context of HCV infection, the downregulation of DNA repair enzyme endonuclease VIII-like 1 (NEIL1) further compromises the cell to remedy oxidative lesions, favoring mutation accumulation [78].

In addition to direct genetic mutations, ROS induces epigenetic changes by altering DNA methylation and histone modification patterns. Long-term oxidative stress can lead to abnormal methylation in the promoter regions of tumor suppressor genes, silencing their expression, or globally lower methylation that contributes to genomic instability. These epigenetic shifts can deregulate tumor-related genes, setting the stage for malignant transformation [79]. ROS-driven genetic alterations frequently affect key tumor suppressor and driver genes. Mutations in TP53, the telomerase reverse transcriptase (TERT) promoter, and catenin beta 1 (CTNNB1) are common in HCC. When these mutations occur, they grant mutant clones a selective growth advantage, enabling them to evade normal cell-cycle checkpoints and apoptotic signals [80–83].

Notably, the mutagenic potential of ROS is further amplified by viral proteins from HBV and HCV. HBx protein disrupts mitochondrial integrity, thereby boosting ROS production, and interferes with p53 function, hampering both DNA repair and apoptosis [84]. Similarly, HCV core proteins induce mitochondrial dysfunction and activate oxidative stress pathways including NF-κB and MAPK, which not only promote inflammatory cytokine production but also alter the expression of genes involved in oxidative stress response and DNA repair [83]. These viral effects further compromise genome integrity, enabling the survival and proliferation of damaged, mutant cells.

Over time, the persistent oxidative assault leads to a gradual buildup of mutations, while simultaneous epigenetic modifications and perturbations of key signaling pathways further destabilize cellular function. Collectively, these changes drive the transformation of normal liver cells into malignant ones.

HCC tumorigenesis

The accumulation of ROS drives the malignant transformation of liver fibrosis and cirrhosis toward HCC, also having a significant impact in the initiation of HCC [85]. As early as the 1980s, experimental evidence from ROS-induced fibroblast transformation in mice highlighted the importance of oxidative stress in carcinogenesis, solidifying its role as a critical factor in HCC initiation and progression [86]. During HCV infection, ROS induce the overexpression of 24-dehydrocholesterol reductase (DHCR24), which binds to p53 and accelerates its ubiquitination and degradation, thereby contributing to the development of HCC [87, 88].

Following malignant transformation, ROS promote HCC progression by activating growth factors through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) and MAPK/extracellular signal-regulated kinase (ERK) mitogenic signaling cascades. Specifically, dysregulated ROS activity leads to the inactivation and oxidative modification of key signaling molecules, such as phosphatase and tensin homolog (PTEN) and protein tyrosine phosphatase 1B (PTP1B), which impairs their ability to inhibit PI3K and results in the hyperactivation of AKT and mTOR [67, 89]. Furthermore, the accumulation of ROS triggers the activation of apoptosis signal-regulating kinase 1 (ASK1), protein kinase G (PKG), and c-Jun N-terminal

kinase (JNK), which, in turn, propagate downstream signaling events in the MAPKK and MAPK mitotic cascades [90]. Moreover, aberrant expression of dynamin-related protein 1 (Drp1) amplifies mitochondrial fission and expedites the G1/S phase transition. Drp1 also mediates ROS-dependent activation of the NF-κB pathway, resulting in p53 inactivation, apoptosis inhibition, and increased resistance to cell death, thereby promoting the abnormal proliferation of HCC cells [91]. In summary, elevated ROS levels in tumor cells promote HCC growth.

HCC metastasis

The effects of ROS extend beyond hepatoma cells, damaging normal cells and tissues and facilitating invasion and adhesion processes. Cancer cells secrete H_2O_2 into neighboring fibroblasts and other stromal cells, mimicking the effects of hypoxia under aerobic conditions and leading to excessive ROS production [92]. Consequently, oxidative stress induced in hepatoma cells is transferred to cancer-associated fibroblasts through H_2O_2. Excessive ROS production in the stroma activates antioxidant defenses in adjacent cancer cells, protecting them from apoptosis, which suggests that tumor cells increase their metastatic potential by raising the threshold for anoikis [93]. Epithelial-to-mesenchymal transition (EMT) is a pivotal early event in HCC metastasis, marked by the loss of intercellular adhesion and reduced interactions with the extracellular matrix (ECM). This process enables tumor cells to acquire migratory capacity toward blood and lymphatic vessels and is driven by aberrant intracellular ROS accumulation [94]. In response, Nrf2 translocates to the nucleus, where it binds to the Notch receptor 1 (Notch1) promoter region, enhancing Notch1 transcription and thereby promoting EMT in HCC, which maintains mesenchymal properties and increases tumor cells adhesion [95]. Furthermore, ROS acts as a second messenger in gene regulation and signal transduction pathways, upregulating factors closely associated with cancer cells metastasis, such as matrix metalloproteinases (MMPs), adhesion molecules, epidermal growth factor (EGF), EGF receptors (EGFR), and vascular endothelial growth factor (VEGF), to facilitate metastasis [96, 97]. In brief, abnormal ROS in hepatoma cells promotes HCC metastasis through multiple pathways. Overall, ROS exacerbate viral infection-induced chronic hepatitis, contribute to liver fibrosis and cirrhosis, and promote HCC growth and metastasis (Fig. 3).

Cell fate

Cell proliferation

As mentioned, ROS significantly influence the regulation of cellular proliferation in HCC development. Specifically in HBV-related HCC, ROS induced by the HBx protein oxidize and deactivate the phosphatases PTEN and PTP1B, thereby weakening their suppression of PI3K/AKT signaling. This disruption leads to PI3K activation, increased phosphatidylinositol 3,4,5-trisphosphate (PIP3) production, AKT phosphorylation, and p53 degradation, collectively promoting tumor cell survival and proliferation [98]. Furthermore, ROS-induced MAPK p38 activation protects hepatoma cells from lysosomal damage-induced, caspase-independent cell death, thereby promoting recovery of lysosomal integrity [99]. Moreover, ROS induce uncontrolled cell proliferation through

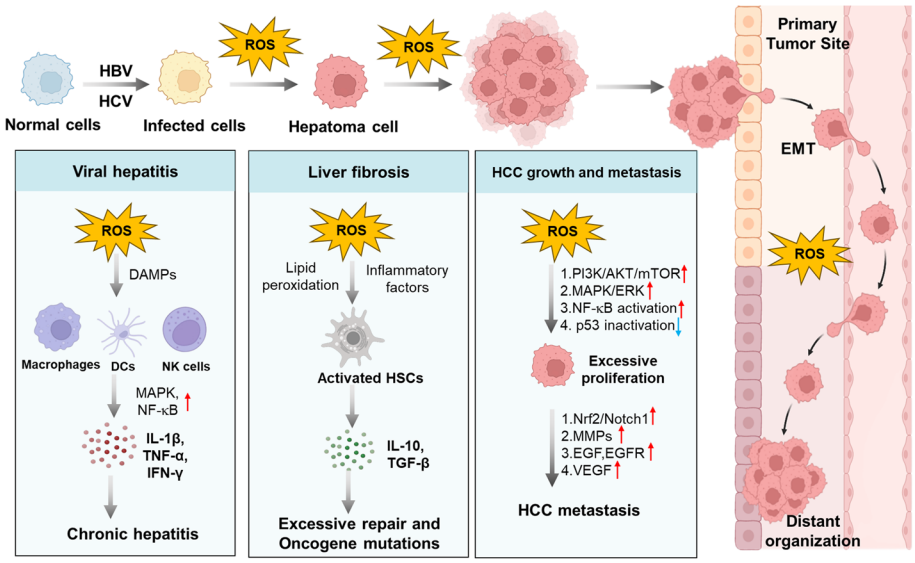


Fig. 3 The temporal heterogeneity of ROS in the malignant transformation from chronic hepatitis to HCC. Upon hepatitis virus infection, the intracellular ROS levels in hepatocytes increase, which triggers the activation of DAMPs and induces the release of inflammatory cytokines such as IL-1β, TNF-α, and IFN-β via the MAPK and NF-κB signaling pathways, promoting the establishment of a chronic inflammatory microenvironment. Persistent ROS generation drives HSCs to transition from a quiescent to an activated state, secreting TGF-β and IL-10, leading to excessive extracellular matrix deposition and liver fibrosis. Simultaneously, M2-polarized macrophages also secrete TGF-β and IL-10, further accelerating collagen deposition and fibrogenesis. At later stages, sustained oxidative stress contributes to the accumulation of DNA mutations and epigenetic alterations, facilitating malignant transformation of hepatocytes. Following transformation, ROS activate oncogenic pathways including PI3K/AKT/mTOR, MAPK/ERK, and NF-κB, while simultaneously inactivating tumor suppressor p53, thereby promoting tumor growth and apoptosis resistance. In the metastatic phase, elevated ROS levels induce EMT through Nrf2/Notch1 pathway activation, allowing hepatoma cells to maintain mesenchymal phenotypes, enhance antioxidant defenses, resist anoikis, and acquire migratory potential toward vascular and lymphatic systems. In parallel, ROS act as second messengers to upregulate metastasis-related molecules, including MMPs, adhesion molecules, EGF, EGFR, and VEGF, thereby facilitating invasion and metastasis. ROS, reactive oxygen species; HCC, hepatocellular carcinoma; DAMPs, damage-associated molecular patterns; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha; IFN-β, interferon beta; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-B; HSCs, hepatic stellate cells; TGF-β, transforming growth factor-beta; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; ERK, extracellular signal-regulated kinase; Nrf2, nuclear factor erythroid 2-related factor; EMT, epithelial-mesenchymal transition; MMPs, matrix metalloproteinases; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; p53, tumor protein 53. (Image created with BioRender.com, with permission)

genomic alterations, such as DNA modifications, which further exacerbate cancer cell invasion and metastatic potential [79, 100].

Cell phenotype remodeling

In the development of viral hepatitis toward HCC, ROS have been shown to significantly influence cell phenotype remodeling. Elevated ROS levels potentially activate signaling pathways such as NF-κB and activator protein-1 (AP-1) in chronic hepatitis, leading to varied cellular responses and functional regulations, such as the M1 polarization of macrophages. Specifically, activation of NF-κB and AP-1 signaling pathways induces key pro-inflammatory cytokines including TNF-α and IL-1β, thereby modulating inflammation and immune responses [101, 102]. Furthermore, HSCs,

the resident mesenchymal cells in the liver, are also activated by ROS in viral hepatitis, transforming into myofibroblasts and promoting liver fibrosis and tumor progression [103]. Moreover, ROS regulation mechanisms in EMT have been extensively studied. Mechanistically, ROS promote HCC migration capabilities by regulating EMT via Ras homologous (Rho) GTPase-dependent mechanisms and matrix degradation via MMPs [104, 105]. Likewise, activation of Nrf2 by ROS not only robustly induces antioxidant genes but also directly influences the Notch signaling pathway, which is essential for maintaining the cancer stem cell characteristics in $CD90^+$ cells [106]. Additionally, Rad51-functional immature hepatocytes proliferate vigorously in a ROS-related chronic inflammatory microenvironment, triggering ductal reaction and transdifferentiation of cholangiocytes into hepatocytes, thereby increasing malignancy and ultimately leading to HCC [107].

Cell senescence

ROS are indispensable in determining cell senescence in HCC development [46]. In HBV-related chronic hepatitis, ROS trigger DNA double-strand breaks and activate the ATM signaling pathway, which in turn activates aging-related pathways such as p53/p21 and ASK1/JNK/p38, promoting the malignant transformation of normal cells [108, 109]. Specifically, activation of oncogene *Ras* promotes excessive cell proliferation by upregulating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, triggering DNA damage repair activation and the aging process [110]. On the other hand, increased ROS production induces mitochondrial dysfunction associated with senescence, leading to reduced respiratory coupling, activation of inflammatory pathways such as NF-κB, and acceleration of the senescence process and HCC development [111]. Conversely, the downregulation of sirtuin 6 (SIRT6) in hepatoma cells enables TGF-β1/ROS to induce cell senescence, counteracting its tumorigenic effects [112].

Cell death

ROS are closely associated with various forms of cell death, including apoptosis, autophagy, ferroptosis, pyroptosis, and necroptosis. Excessive levels of ROS exhibit a direct positive correlation with liver cell apoptosis and elicit mitochondrial-dependent apoptotic pathways by influencing mitochondrial function and structural integrity in HCC development. In the endogenous apoptotic pathway, ROS provoke the release of cytochrome c from the mitochondrial membrane into the cytoplasm, where it subsequently associates with apoptosis-inducing factor 1 (Apaf-1), culminating in the formation of apoptotic bodies [113]. In the exogenous apoptotic pathway, excess ROS instigates the binding of death receptors and cognate ligands on the cellular membrane, thereby activating the intracellular components of the death receptors. This leads to the interaction with Fas-associated death domain protein (FADD), ultimately giving rise to the formation of lethal signaling complexes [114]. Apoptosis signals activated by ROS subsequently triggers the recruitment and activation of the caspase cascade, leading to chromatin fragmentation and hepatoma cell death.

Previous studies have shown that multiple mechanisms link ROS to the initiation of autophagy in viral hepatitis-induced HCC [115]. H_2O_2, for instance, exerts its influence by inhibiting autophagy-related protein 4 (ATG4), thereby bolstering lipidated

microtubule-associated protein 1 light chain 3-II (LC3-II levels), which facilitates LC3-II binding to autophagosomes and promotes p62 oligomerization, enhancing autophagosome biogenesis [116, 117]. Furthermore, p62-mediated Nrf2 activation hampers autophagy in the liver under stress, instigating Nrf2-mediated metabolic reprogramming and cell cycle events linked to hepatocarcinogenesis [118].

Ferroptosis is a form of nonapoptotic cell death dependent on iron and ROS, exerting a negative regulatory effect on HCC initiation and progression [119]. A key characteristic of ferroptosis is the peroxidation of polyunsaturated fatty acids in cells, which is caused by high levels of intracellular iron and ROS-induced lipid peroxidation [120]. The nonclassical pathway of ferroptosis is mainly regulated by Nrf2/Keap1 signaling, and is one of the most common mutation pathways in HCC [121].

Pyroptosis, a programmed cell death marked by cell expansion, rupture, and the release of inflammatory cytokines, is critically regulated by ROS [122]. ROS promotes the formation of caspase-1, caspase-3, and caspase-4/5, which generate active N-terminal domains through the hydrolysis of Gasdermin D (GSDMD), ultimately leading to hepatoma cell membrane perforation and rupture [123].

Necroptosis, a caspase-independent form of cell death with characteristics intermediate between apoptosis and necrosis, is positively correlated with elevated ROS [124]. ROS promotes receptor interacting serine/threonine kinase 3 (RIP3) phosphorylation, which subsequently triggers downstream mixed lineage kinase domain-like pseudokinase (MLKL) phosphorylation and activates MLKL translocation to the plasma membrane, increasing membrane permeability and inducing necrotic death in HCC tumor cells [125].

The spatial heterogeneity of ROS in the malignant transformation of viral hepatitis to HCC

The spatial heterogeneity of ROS is demonstrated by its varying reactivity across different liver cell types, subcellular structures, and molecules in the progression of viral hepatitis-induced HCC. Notably, the function, activity, and survival of liver cells are regulated by redox reactions, involving both intracellular and extracellular ROS and cellular antioxidants. The differing reactivity of liver cells to ROS can be attributed to distinct antioxidant systems within these cells, notably the GSH and Nrf2 signaling pathways. In contrast, the weaker antioxidant capacity of normal cells is partly due to the influence of oncogenes such as k-Ras and c-Myc, which stabilize Nrf2 in tumor cells, thereby enhancing their survival [126]. In addition, ROS induce DNA and RNA damage, and lipid and protein peroxidation, which lead to endoplasmic reticulum stress, as well as mitochondrial and lysosomal dysfunction, thereby promoting the initiation and progression of HCC. The following sections will primarily explore the regulatory effects of ROS on various liver cells, organelles, and molecules (Fig. 4A–C).

Liver cells

Hepatocytes

Hepatocytes are a primary site for ROS production and are highly sensitive to ROS-induced damage [69]. Oxidative stress promotes calcium influx into cells and redistributes intracellular calcium from the endoplasmic reticulum (ER) to the cytoplasm,

mitochondria, and nucleus, which increases mitochondrial permeability transition, facilitates the release of pro-apoptotic factors such as cytochrome c, and activates calcium-dependent nucleases, proteases, and lipases [127, 128]. This causes the death of hepatocytes, which activates DAMPs and subsequent chronic inflammation induced by macrophages and natural killer cells (NK cells) [69]. Additionally, ROS induce genomic changes in hepatocytes, contributing to cellular malignancy [129]. Additionally, ROS impair the secretory function of hepatocytes by disrupting bile flow formation, leading to cholestasis and fibrosis [130]. In summary, hepatocytes malignancy and chronic inflammation induced by hepatocyte death promotes the development of HCC.

HSCs

ROS play a critical role in the differentiation and activation of HSCs into myofibroblasts, which in turn leads to the secretion and accumulation of collagen and other ECM components in the liver [131]. HSC activation is triggered by several factors, including ROS release and inflammatory mediators from damaged liver cells, activation of inflammatory cells such as macrophage to secrete pro-fibrotic cytokines, and lymphocyte infiltration into the injured site. ROS-induced TGF-β is the most potent fibroblast-activating factor, activating HSCs in a mothers against decapentaplegic homolog 2/3 (SMAD2/3)-dependent manner and directly inducing the transcription of collagen alpha-1 (COL1A1) and COL1A2 [132]. Under pathological conditions, cytokines such as IL-33 are released from stressed liver cells to activate HSCs

(See figure on next page.)

Fig. 4 The spatial temporal heterogeneity of ROS in the malignant transformation from chronic hepatitis to HCC. **A** Different cell types in the liver microenvironment exhibit distinct responses to ROS during the progression from chronic hepatitis to HCC. In the hepatitis stage (black font), hepatocytes suffer ROS-induced injury, activating DAMPs and triggering chronic inflammation via immune cells (T cells, B cells, NK cells, macrophages, and DCs). ROS also promote HSC activation and fibrogenesis. In the HCC stage (red font), hepatoma cells maintain elevated ROS levels while enhancing antioxidant systems (GSH, Nrf2) to support proliferation, metastasis, and immune escape. Simultaneously, ROS reshape immune cell functions, suppressing T cell cytotoxicity, impairing NK cell surveillance, and altering macrophage polarization, thereby creating a tumor-permissive microenvironment. **B** Different organelles respond to ROS damage at various stages of liver carcinogenesis. Mitochondria act as both producers and targets of ROS, where excess ROS induce mtDNA mutations, metabolic dysfunction, and apoptosis resistance. ER stress, triggered by ROS accumulation, amplifies viral replication and activates oncogenic signaling cascades. Lysosomes undergo ROS-induced rupture, impairing autophagic flux and promoting inflammation. Liver microsomes are subject to ROS-driven lipid peroxidation and cytochrome P450 damage, exacerbating metabolic reprogramming, drug resistance, and malignant transformation. **C** Various biomolecules are vulnerable to ROS-induced modifications. DNA damage involves base oxidation (8-OHdG), strand breaks, telomere shortening, and mutagenesis, facilitating HCC initiation. RNA is affected via base oxidation and epigenetic alterations such as m^6A modification, altering mRNA stability and translation in favor of tumor progression. Lipid peroxidation generates reactive byproducts (HNE, MDA) that disrupt membrane integrity and activate inflammatory pathways. Protein modifications, including carbonylation, phosphorylation, and ubiquitination, regulate oncogenic signaling, immune evasion, and cell death during hepatitis-driven HCC development. ROS, reactive oxygen species; HCC, hepatocellular carcinoma; DAMPs, damage-associated molecular patterns; NK cells, natural killer cells; DCs, dendritic cells; HSCs, hepatic stellate cells; GSH, glutathione; Nrf2, nuclear factor erythroid 2-related factor 2; ER, endoplasmic reticulum; mtDNA, mitochondrial DNA; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; m^6A, N^6-methyladenosine; HNE, 4-hydroxynonenal; MDA, malondialdehyde. (Image created with BioRender.com, with permission)

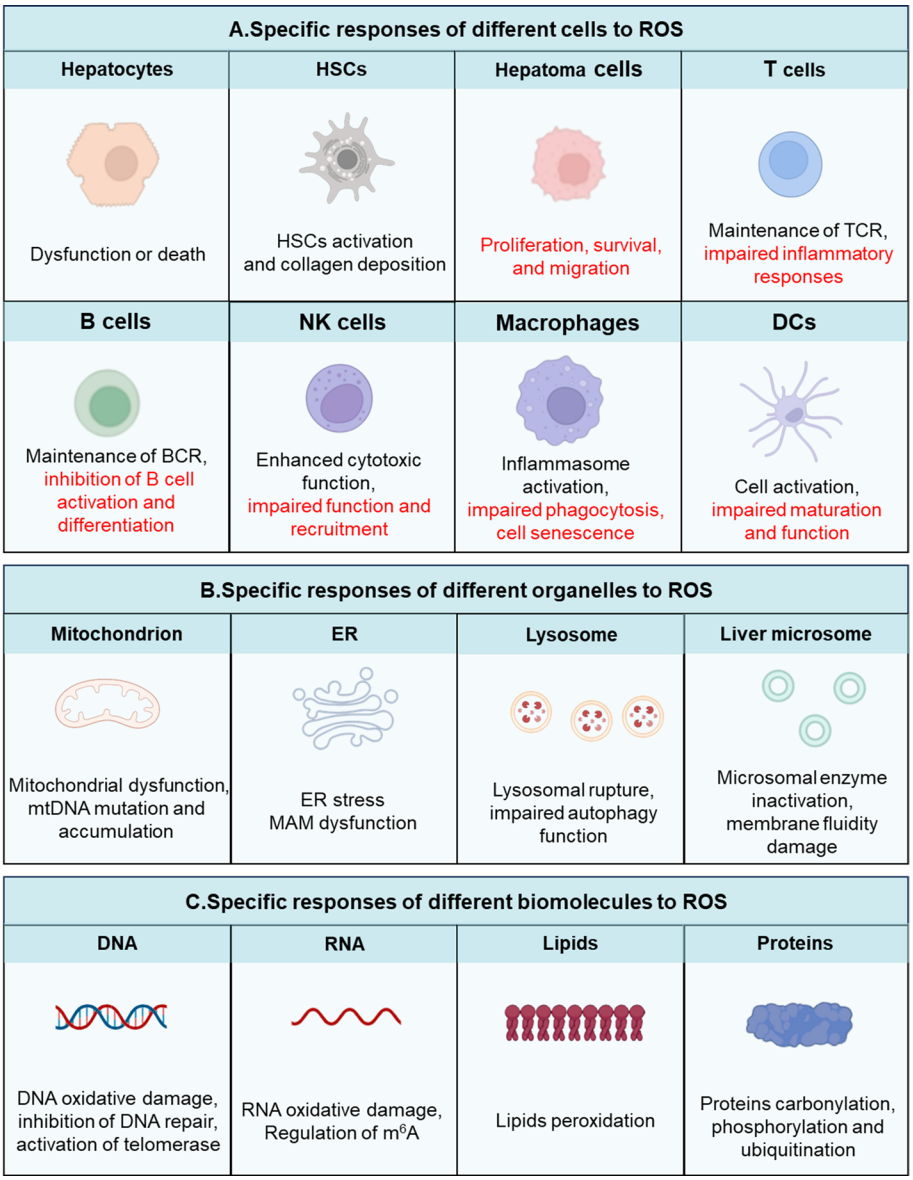


Fig. 4 (See legend on previous page.)

and promote fibrosis [133]. Consequently, prolonged HSC activation due to oxidative stress contributes to liver fibrosis, which can progress to cirrhosis and HCC [131]. Additionally, tumor-specific HSCs activate the Nrf2–Keap1 pathway in dendritic cells (DCs), resulting in increased ROS production, which inhibits the expression of signaling molecules such as CD80, CD86, and IL-12, thereby suppressing splenic T cell activation and promoting HCC onset and progression [134].

Hepatoma cells

As mentioned, hepatoma cells induce excessive production of ROS [135]. To mitigate the excessive ROS production and its associated toxic effects while sustaining cell proliferation and survival, hepatoma cells activate two primary response mechanisms: GSH

metabolism and the Nrf2–Keap1 pathway [136]. During hypoxia, large quantities of ROS and NO are generated, and glutathione plays a key role in maintaining intracellular redox homeostasis. Glutathione reductase (GRd) converts oxidized glutathione (GSSG) back into its reduced form (GSH) by obtaining electrons from NADPH, which neutralizes and reduces the damage caused by ROS [137]. Additionally, Nrf2 overactivation in hepatoma cells inhibits the pro-inflammatory response by suppressing cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression, creating a favorable environment for malignant cell survival and protecting them from oxidative stress [138]. In summary, hepatoma cells exhibit elevated levels of ROS, accompanied with the activation of antioxidant signaling pathways that mitigates the cytotoxic effects typically induced by excessive ROS.

T cells

In chronic hepatitis, ROS produced by oxidative phosphorylation (OXPHOS) enhance and maintain T cell signaling. At low levels, ROS induce nuclear factor of activated T cells (NFAT), which supports metabolic changes by regulating the transcription of hypoxia-inducible factor-1 alpha (HIF-1α) [139]. Additionally, low ROS concentrations stabilize HIF-1α and activate mechanistic target of mTOR, promoting IL-17-producing T helper (Th17) cell differentiation, which increases IL-17A production and inhibits regulatory T cells (Tregs) cell proliferation and differentiation [140–142]. Concurrently, ROS produced by mitochondria activate the AMPK pathway, facilitating the differentiation of $CD8^+$ T cells and exacerbating the severity of chronic inflammation [143]. Persistent HBV-related chronic hepatitis induces T cell inactivation and subsequent death, whereas antioxidant therapy in mice restores T cell proliferation during HBV infection [144]. However, excessive ROS signaling in T cells during HCC progression leads to metabolic disorders and impaired inflammatory responses, hindering $CD8^+$ T cell differentiation and reducing the Th17/Treg ratio [145, 146].

B cells

Research has shown that the loss of function of memory B lymphocytes is closely related to the persistence of inflammation after HBV infection and the development of HCC [147]. Elevated levels of H_2O_2 are crucial for initiating and maintaining B cell receptor (BCR) signaling during HCC progression, primarily derived from mitochondrial ROS [148]. Cellular redox status and mitochondrial ROS release are essential for B cell survival, differentiation, and immunoglobulin M (IgM) synthesis [149]. However, excessive ROS synthesis can inhibit B cell activation and differentiation into antibody-producing plasmablasts [150]. Besides, prolonged elevation of ROS levels causes increased consumption of IgM antibodies and suppresses antibody production by downregulating CD19 expression [151, 152]. In summary, increased ROS levels inhibit B cell function and promote the development of HCC.

NK cells

ROS play a critical role in NK cell-mediated cytotoxicity and the proliferation following HBV and HCV invasion [153]. HBV or HCV infection significantly increases the expression of interferon-gamma (IFN-γ) expressed by NK cells accompanied with

ROS increase in the liver, while the induction of IFN-γ decreases after the use of NK cell function inhibitors [154]. Moreover, ROS-induced activation of Nrf2 functions as an immune checkpoint following NK cell activation [155]. Upregulation of GSH synthesis may enhance NK cell proliferation and cytotoxic function in HCC [156]. Conversely, downregulation of GSH impairs NK cell function and their recruitment to inflammatory sites [156]. In inflammatory environments, upregulation of Nrf2 and GSH protects NK cells from damage caused by H_2O_2 [157]. However, oxidative stress impairs the function of NK cells in the context of persistent chronic hepatitis and HCC. Compared with healthy individuals, patients with HBV-induced HCC exhibit reduced NK cell cytotoxicity and decreased production of IFN-γ and TNF-α [158]. Mechanistically, ROS-activated mitochondrial fission reduces NK cell number and cytotoxicity, enabling tumor cells to evade NK cell-mediated surveillance, thereby promoting recurrence and metastasis of HCC [159]. Further investigations reveal that hypoxia-mediated excessive activation of the mTOR pathway induces phosphorylation of Drp1 at the Ser616 site, which causes excessive mitochondrial fragmentation and apoptosis in NK cells [160].

Macrophages

Macrophage ROS levels influence the activities of signal transducer and activator of transcription 1 (STAT-1), MAPK, and NF-κB and activate the NLR family pyrin domain containing 3 (NLRP3) inflammasome, enhancing inflammatory signaling in the progression from hepatitis to HCC [85, 161]. Kupffer cells (KCs), the resident macrophages of the liver, play a significant role in liver homeostasis and disease. Autophagy-deficient Kupffer cells exacerbate liver fibrosis, inflammation, and the development of HCC during the precancerous stage by enhancing the mitochondrial ROS–NF-κB–IL1α/β pathway [162]. Additionally, cellular stressors such as ultraviolet rays (UV) irradiation and hypoxia induce the expression of cold shock protein Cirp, increasing IL-1β and IL-6 production in KCs [163, 164]. Additionally, ROS levels impact the assembly of NADPH oxidase subunits, affecting H_2O_2-mediated intracellular signaling and causing macromolecular damage [165]. Sustained high levels of ROS or NO promote macrophage senescence through sustained expression of IL-10 and TGF-β [85, 166].

In addition, macrophage function and polarization patterns are influenced by GSH levels and Nrf2 activity in HCC development [167]. The GSH system is important for regulating the M1 inflammatory state and the production of prostaglandin E2 (PGE2) and NO, while protects macromolecules from oxidative damage [167]. The antiviral response initiated by M1 macrophage activation, including increased expression of STAT-1, interferon regulatory factor (IRF) 7, and IRF9, also depends on the GSH system [168]. Additionally, Nrf2 upregulation plays an anti-inflammatory role in activated macrophages by attenuating the activities of IL-1β and IL-6 and increasing CD163 and arginase-1 (Arg1) expression [168, 169].

DCs

Substantial evidence suggests that DC dysfunction is prevalent in chronic liver inflammation and HCC, closely related to oxidative stress [170]. In the early stages of infection, hepatitis viruses activate Toll-like receptors (TLRs), which in turn stimulate the activation of DCs. ROS produced by DCs following TLR activation play a pivotal role in

the maturation and priming of $CD4^+$ T cells, which accelerates the formation of chronic hepatitis [170]. However, excessive ROS hinder DC maturation and impair function in chronic viral hepatitis, contributing to viral persistence. Mechanistically, oxidative stress impairs DCs ability to stimulate species-specific markers (CD83, CD86, and CD40) and is negatively correlated with programmed cell death-ligand 1 (PD-L1) expression [171, 172]. Furthermore, ROS-associated transcription upregulates expression of exonucleases CD39 and CD73 in HCC cells, facilitating recruitment of pDCs to tumors via the adenosine A1 receptor (ADORA1) and thereby exacerbating HCC progression [173]. Disruption of redox homeostasis impairs DC maturation and reduces inflammatory cytokine production, contributing to severe HCC progression [174].

Organelles

Mitochondrion

Mitochondria are the primary ROS production site within cells and the main target organelle of ROS attack and damage as well in HCC development [175]. In viral hepatitis, the HBx protein targets and binds to the outer mitochondrial membrane, resulting in elevated ROS levels [176]. Concurrently, the HBx protein interacts with cytochrome c oxidase III (COXIII), impairing adenosine triphosphate (ATP) synthesis [177]. Additionally, HBV infection also upregulates the expression of unidirectional transport proteins, increasing mitochondrial calcium ion uptake, which enhances membrane permeability, making cells more sensitive to ROS [178]. Likewise, HCV can stimulate the mitochondrial cytochrome P450 family 2 subfamily E member 1 (CYP2E1), inducing ROS generation and the production of lipid peroxide products [179].

Excess ROS motivate mitochondrial permeability transition pore (mPTP) opening, causing mitochondrial swelling, rupture and cytochrome c release, and ultimately cell death and chronic inflammation [180]. Under oxidative stress, mitochondria consume large amounts of GSH, lowering antioxidant defenses. Excessive GSH consumption in mitochondria makes hepatocytes more susceptible to TNF-α-induced cell death, which results in chronic hepatitis and canceration [181]. In addition, mtDNA is susceptible to oxidative stress, contributing to DNA breakage and somatic mutations in the mtDNA molecule owing to its proximity to the ETC, absence of protective histones, and insufficient repair mechanisms [182]. Accumulated mtDNA mutations are considered vital in the progression from hepatitis to HCC [176]. In summary, viral-induced ROS increase promotes cell malignancy and accelerates the development of HCC by inducing mitochondrial dysfunction and accumulating mtDNA mutations.

Endoplasmic reticulum

ROS-induced ER stress worsens the severity of hepatitis and may even induce HCC. The presence of multiple HBV proteins causes surface protein accumulation in the ER, triggering the unfolded protein response (UPR) and creating a vicious cycle of chronic hepatitis and oxidative stress [183]. Mechanistically, HBx-mediated activation of the activating transcription factor 6 (ATF6) and inositol requiring enzyme 1α (IRE1α)-X-box binding protein 1 (XBP1) pathways facilitate HBV replication and expression in hepatic cells, thereby exacerbating hepatitis [184]. Similar to HBV, HCV infection also triggers the UPR, resulting in the generation of ROS and activation of UPR and NF-κB signaling

pathways [185]. The activated NF-κB translocates to the nucleus and upregulates the expression of TGF-β1, which paradoxically inhibits acute HCV replication, mitigates HCV cytotoxic effects, and fosters the establishment of chronic HCV infection [186]. On the other hand, multiple factors in hepatitis trigger the accumulation of lipid and protein peroxides and DNA mutation products and prompt ER Ca^{2+} leakage into the cytoplasm, increasing ROS production in mitochondria [187].

The involvement of ROS in regulating crosstalk between the mitochondrial-associated endoplasmic reticulum (MAM) and its influence on homeostasis and hepatic malignancy function is also noteworthy [188]. Of particular relevance, the glucose-regulated protein 75 (GRP75), a catalyst in MAM formation, exerts control over ROS production and the activity of antioxidant systems, as evidenced through its modulation of MMPs, Nrf2, and Nqo1, thereby culminating in redox imbalance. In parallel, GRP75 assumes a pivotal role in the regulation of key factors, encompassing HIF-1α, p-Akt, and c-Myc, engendering alterations in metabolic reprogramming that invariably foster the growth of HCC cells [188]. In summary, the vicious cycle of ER stress and oxidative stress alters ROS production and clearance, exacerbating the process from hepatitis to HCC.

Lysosome

ROS has been identified as a causative agent in lysosomal rupture, a phenomenon typically associated with the later stages of necrosis [189]. Lysosomal compartments, enriched with iron from prominent molecules such as ferritin and mitochondrial metalloproteins, are susceptible to instability induced by lipid peroxidation [190]. Specifically, oxidative stress induced by HBV activates PRKAA (the catalytic subunit of AMPK), enhancing autolysosome-dependent degradation by increasing cellular ATP levels, which depletes autophagic vacuoles [191]. Furthermore, the escalated levels of ROS induced by HCV prompt an induction of autophagy, a crucial mechanism ensuring the effective replication, morphogenesis, and release of infectious viral particles [192]. However, the increased expression of p62 in HCV-related HCC indicates the presence of autophagy defects in human HCC [193]. Upon consumption of a high-fat diet, the oxidation of free fatty acids in mitochondria produces ROS, which exacerbates lysosomal damage by promoting calcium protease-mediated carbonylation of Hsp70.1 cleavage [194]. Damaged lysosomal function impairs autophagy, exacerbating viral hepatitis and potentially contributing to HCC onset and progression.

Liver microsome

ROS are closely related to the structure and function of liver microsomes. ROS generated during viral hepatitis attack the lipids in liver microsomal membranes, causing lipid peroxidation that damages membrane structure and function, impacting membrane fluidity and enzyme activity within the microsomes [195]. Furthermore, ROS oxidize proteins in liver microsomes, including those in the CYP450 enzyme system, which results in the loss or alteration of enzyme activity [61]. CYP450 within liver microsome promotes ROS production and contributes to HCC development, a process inhibited by nuclear protein 1 (NUPR1). Mechanistically, NUPR1 interacts with the aromatic hydrocarbon receptor

(AhR), leading to AhR degradation via the autophagy–lysosome pathway and reducing nuclear translocation, thereby impairing CYP transcription [196]. In summary, ROS-related liver microsomal damage promotes the occurrence and drug resistance of HCC.

Biomolecules

DNA

Hepatitis virus infection induces hepatic oxidative stress, amplifying ROS production, which in turn causes DNA oxidative damage and exacerbates liver inflammation. Patients with chronic HBV infection exhibit an accumulation of the DNA damage product 8-OHdG [73]. Additionally, HBV inhibits DNA repair pathways following oxidative damage, increases genotoxic intermediates, and promotes HBV genome integration, thereby enhancing liver inflammation [197]. Chronic HCV infection is associated with increased oxidative stress, ROS generation, and oxidative DNA damage [76]. Similar to HBx, HCV core protein inhibits the repair of oxidative damage by inhibiting the activity of DNA glycosylases that repair 8-OHdG. Moreover, HCV proteins also interact with multiple DNA repair factors, negatively regulating the repair of DNA oxidative damage, leading to increased levels of 8-OHdG and single-strand breaks in individuals infected with HCV [198, 199].

Oxidative DNA damage caused by ROS during hepatitis increases the risk of DNA mutations, which accumulate and contribute to the development of HCC. Besides, ROS exert a regulatory influence over telomeric dynamics, a pivotal determinant entwined with cell proliferation, senescence, and carcinogenesis [200]. Empirical investigations have illuminated the propensity of oxidative stress to expedite the erosion of telomeric sequences [201]. Concurrently, within the intricate mosaic of hepatocarcinogenesis, the revival of telomerase assumes an indispensable mantle, which unfurls as a requisite instigator for the orchestrated surge of cell proliferation, engendering a poignant metamorphosis toward malignant transformation and culminating in the emergence of HCC [202]. In summation, the intricate choreography of oxidative stress, coalescing with the symphony of DNA damage, constitutes a decisive motif in the multifaceted panorama of HCC pathogenesis.

RNA

In addition to inducing DNA oxidative damage, ROS also precipitate RNA oxidative damage in the context of chronic hepatitis. Owing to its single-stranded structure, RNA is inherently more vulnerable to ROS, leading to base mutations or single-strand breaks. In HBV-induced chronic hepatitis, the concentration of the RNA oxidation product 8-oxo-7,8-dihydroguanosine (8-oxoGsn) is positively correlated with the severity of liver injury [203].

Furthermore, ROS, an intracellular signaling molecule influencing the epigenetic modification of RNA, is most commonly found in N^6-methyladenosine (m^6A) in the malignant transformation from viral induced chronic hepatitis to HCC [204]. Methyltransferase-like 3 (METTL3) is an *S*-adenosylmethionine (SAM) binding protein facilitating the transfer of methyl groups from SAM to adenine bases in RNA [205]. Notably, METTL3 is positively regulated by ROS and its interaction with hepatitis B X-interacting protein (HBXIP) induces HIF-1α methylation of m^6A, promoting metabolic

reprogramming that supports liver malignancy [206]. Conversely, the downregulation of METTL3 mitigates glycolysis and invasiveness in HCC cells [206]. The Reader YTH domain-containing family protein 1 (YTHDF1), when depleted, inhibits the proliferation, migration, autophagy, and cell cycle processes of HCC cells [207]. Under hypoxia-induced ROS accumulation, YTHDF1 enhances the translation of autophagy-related genes (ATG2A and ATG14) by binding to m^6A-containing mRNA, thereby promoting HCC cell proliferation via the PI3K/AKT/mTOR signaling pathway [208]. In summary, ROS regulate the levels of m6A in RNA, exacerbating the progression of hepatitis and facilitating the onset and development of HCC.

Lipids

ROS initiate lipid peroxidation within hepatocytes, forming aldehyde byproducts such as HNE and MDA [209]. Lipid peroxidation (LPO) induces apoptosis and ferroptosis by directly modifying intracellular NF-κB and JNK signaling pathways, and triggers cellular inflammation by interacting with pro-inflammatory cell receptors or kinases in liver injury [210]. LPO is also a key factor in HCV replication, where the membrane-proximal domains of NS3/4A and NS5B proteins act as LPO sensors in the HCV replicase complex, shutting down replicase activity through conformational changes in response to LPO exposure [211]. Additionally, $CD36^+$ cancer-associated fibroblasts create an immunosuppressive microenvironment that facilitates HCC development through secretion of macrophage migration inhibitory factors (MIF). Mechanistically, this process involves activation of the lipid peroxidation/p38/CCAAT enhancer binding protein (CEBPs) axis, mediating oxidative LDL uptake and promoting MIF expression [212].

Proteins

ROS regulate proteins by various post-translational modifications during the pathophysiology of hepatitis. Protein carbonylation, a key modification during oxidative stress, is pivotal in the progression from hepatitis to HCC [213]. ROS induce hypermethylation of the *Runt-related transcription factor 3 (RUNX3)* gene promoter region, which correlates with increased plasma protein carbonylation and is associated with a higher risk of HBV-related HCC [214]. In addition, ROS also regulates protein phosphorylation and ubiquitination in HCC development. HCV promotes chronic hepatitis by inducing NF-κB phosphorylation and activating the JNK and IRE1 pathways through ROS generation [186]. Furthermore, depletion of peroxiredoxin 4 (PRDX4) in HCC cells causes excessive ROS production, which reduces β-catenin ubiquitination, enhances β-catenin stability, and activates β-catenin signaling, thereby increasing resistance to apoptosis in HCC cells [215].

Treatment strategy for HCC development based on ROS spatiotemporal heterogeneity

Based on the above-mentioned ROS heterogeneity, the following treatment strategies are proposed. In the progression from chronic hepatitis to HCC, the accumulation of ROS inflicts damage on normal cells and exacerbates cellular injury. Therefore, reducing ROS levels during the early stages can be an effective strategy for preventing and treating HCC. However, in advanced HCC stages, where tumor cells have metastasized,

therapeutic approaches often aim to increase ROS levels to induce tumor cell death. While potentially effective against cancer cells, this strategy often causes substantial side effects, including mutations or death of normal cells. Additionally, maintaining normal concentrations of ROS is critical for resisting malignant transformation for immune cells and normal liver cells. However, excessive accumulation of ROS impairs normal cellular functions. Therefore, therapeutic strategies should aim to sustain optimal ROS levels in immune and liver cells while reducing the excessive ROS associated with HCC cells. Simultaneously, drugs should elevate ROS levels specifically in HCC cells to activate cell death pathways and inhibit the progression of HCC (Fig. 5). In addition, as understanding of the role of ROS in the malignant transformation of viral hepatitis to HCC continues to deepen, the development of drug therapies, immunotherapies, and other innovative treatment strategies has expanded therapeutic options, paving the way for more precise and personalized approaches to HCC management.

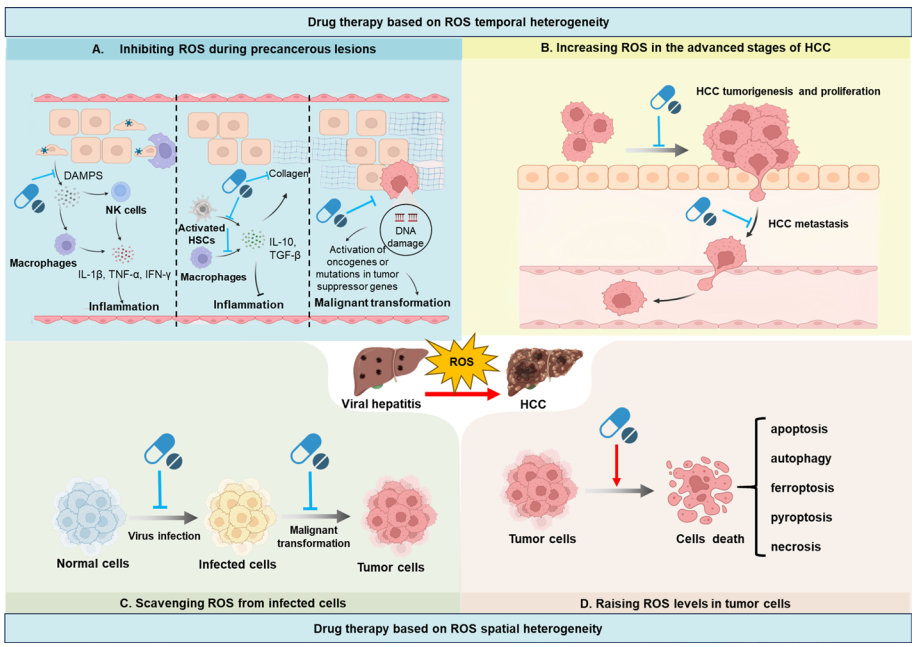


Fig. 5 Therapeutic strategies targeting ROS in HCC malignant transformation by hepatitis based on ROS spatiotemporal heterogeneity. **A** In the early stages of disease, ROS accumulation in hepatocytes and immune cells promotes inflammation, fibrosis, and oncogenic transformation. Therefore, antioxidant-based drugs aim to reduce ROS levels, alleviating chronic inflammation, suppressing hepatic stellate cell activation, and delaying malignant transformation. **B** In advanced HCC stages, excessive ROS can be exploited therapeutically to induce tumor cell apoptosis, autophagy, ferroptosis, pyroptosis, or necroptosis, thereby inhibiting tumor growth and metastasis. Drugs such as sorafenib, lenvatinib, regorafenib, and various natural compounds increase ROS to trigger cancer cell death. **C** At the interface between infection and immune response, appropriate regulation of ROS is critical to maintain antiviral immunity and prevent tissue damage. Antioxidants can help control oxidative stress, protect normal liver and immune cells, and limit viral replication, especially in chronic hepatitis. **D** Conversely, selective elevation of ROS in tumor cells can disrupt redox balance, activate intrinsic and extrinsic cell death pathways, and inhibit cancer progression. ROS, reactive oxygen species; HCC, hepatocellular carcinoma. (Image created with BioRender.com, with permission)

Drug therapies

During the progression from viral hepatitis to HCC, especially in precancerous lesions and early-stage HCC, ROS-induced cell damage can promote the transformation of hepatitis to HCC, impair the immune system, and exacerbate the progression of the disease. Thus, drugs aiming at inhibiting ROS in these stages hold promise for reducing the risk of HCC progression. However, in the advanced stages of HCC, the situation changes. Some drugs can specifically increase ROS generation or inhibit the antioxidant system in tumor cells, which has the potential to kill HCC cells.

Inhibiting ROS to reduce normal cell malignancy during precancerous lesions

ROS-induced cell damage during hepatitis is an important factor in promoting the transformation of hepatitis to HCC. At the same time, its damage to normal cells in the cancer stage can impair immune effects and cause a decrease in liver function, leading to the progression of HCC and the deterioration of the patient's physical function. Therefore, downregulating ROS during viral hepatitis and the early stages of HCC can alleviate inflammation and reduce the risk of HCC progression. Table 1 summarizes potential drugs for treating viral hepatitis and HCC by inhibiting ROS.

Drug-induced reduction of ROS elucidates the mechanisms underlying various treatments for hepatopathy. Many potential therapeutic agents for viral hepatitis and early-stage HCC aim to attenuate ROS levels. The natural product silymarin inhibits lipid peroxidation, reduces the occurrence and growth of liver cancer, and restores the ultrastructure of liver cells [216]. Quercetin reduces oxidative stress, inflammation, hepatic stellate cell activation, and autophagy by inhibiting the TGF-β1/SMAD, PI3K/AKT, and p38 MAPK pathways [217, 218]. Moreover, antiviral therapy has demonstrated significant efficacy in alleviating oxidative stress in patients with viral hepatitis, thereby offering protective benefits for liver function. In patients with chronic hepatitis C receiving peginterferon and ribavirin combination therapy (PegIFN/RBV), serum metabolomics analyses have revealed a marked reduction in ROS levels, with the glutathione metabolic pathway identified as a key mediator in oxidative stress attenuation [219]. In addition to antiviral treatments, branched-chain amino acids (BCAAs) have shown therapeutic potential in patients with liver cirrhosis. Specifically, in HCV transgenic mice and HCV-associated advanced fibrosis patients with iron overload, 48 weeks of BCAA supplementation not only reduced oxidative stress by restoring mitochondrial function but also improved iron metabolism by enhancing the expression of hepcidin-25 [220]. In summary, pharmacological agents that lower ROS levels mitigate the risk of carcinogenesis in normal liver cells, enhance the antioxidant capacity of normal cells, and consequently exert therapeutic effects.

However, in certain cases, the clinical use of some antioxidants has not shown potent anti-HCC effects, especially in the advanced stage of HCC. In a phase I dose-finding study, patients with advanced HCC who were ineligible for other therapies owing to impaired hepatic function were treated with silybin phosphatidylcholine, a potent hepatoprotective antioxidant. However, during treatment, liver function worsened and the tumor marker α-fetoprotein increased in all three patients, and all participants died within 23–69 days of enrolling into the trial, likely from hepatic failure [221]. This failure may be related to the few participants enrolled and the profound loss of liver function in

Table 1 Potential drugs that inhibit ROS during precancerous lesions

Agent	Liver disease	Index changes	Effects	Phase	Refs.
Metformin	Viral hepatitis	MDA↓, TAC↑	Improves liver damage in HCV-infected beta thalassemia major (β-TM) adolescent patients	Prospective cohort study	[223]
Bicyclol	Viral hepatitis	ROS↓	Ameliorates chronic inflammation	Clinical trial	[224]
Silymarin	Viral hepatitis	MDA↓, TAC↑, SOD ↑, GSH↑	Increases the efficacy of DAAs, diminishes latent viral load, and revamps the level of sex hormones	Randomized controlled trial	[216]
Fluoxetine	Viral hepatitis	ROS↓	Reduces the production of ROS and lipid accumulation; activates STAT-1 and JNK signaling for promoting IFN-α-mediated antiviral effects	Clinical trial	[225]
Curcumin	Hepatic fibrosis	ROS↓, MDA↓, CAT ↑, GSH↑, GSH-Px↑, SOD↑	Promotes Nrf2/HO-1 pathway; inhibits NF-κB and TGF-β/SMAD3 pathways	Randomized controlled trial	[226]
Ginsenoside	Hepatic fibrosis	ROS↓, NOX4↓	Reduces NOX4-mediated ROS oxidative stress and inhibits inflammasome activation	Preclinical study	[227]
Oleanolic acid	Hepatic fibrosis	ROS↓, SOD↑, GSH↑, CAT↑	Protects against the activation of HSCs by decreasing the TGF-β1 level and oxidative stress	Preclinical study	[228]
Resveratrol	Hepatic fibrosis	MDA↓, SOD↑, GSH↑, GSH-Px↑	Reduces inflammation and oxidative stress in HSCs by inhibiting ER stress, NF-κB/TNF-α, TGF-β1/Smda3 pathways and activating Nrf2 and miR-20a-mediated PTEN/PI3K/AKT pathways and regulating iron homeostasis	Randomized, double-blinded, controlled clinical trial	[229]
	HCC	ROS↓, GSH↑, SOD↑, GSH-Px↑	Improves oxidative stress by inducing SIRT1/Nrf2 pathway and inhibiting NF-κB pathway	Preclinical study	[230]
Honokiol	Hepatic fibrosis	MDA↓, GSH↑, SOD↑	Suppresses oxidative stress and inflammation; inhibits TGF-β/SMAD/MAPK pathway	Preclinical study	[231]
Berberine	Hepatic fibrosis	ROS↓, MDA↓ GSH↑, SOD↑	Induces ferrous redox to activate RPD-mediated HSC ferroptosis, activates AMPK, and blocks Nox4 and Akt expression	Randomized, double-blind placebo-controlled trial	[232]
Riboflavin	Hepatic fibrosis	MDA↓, SOD↑	Delays hepatic fibrosis by enhancing the mitochondrial function via the AMPK/PGC-1α/HO-1 and MAPK pathways	Randomized clinical trial	[233]

Table 1 (continued)

Agent	Liver disease	Index changes	Effects	Phase	Refs.
Taxifolin	Hepatic fibrosis	MDA↓, SOD↑, GSH↑	Inhibits inflammation, oxidative stress, and apoptosis by regulating PI3K/AKT/mTOR and TGF-β1/SMAD pathways in hepatic fibrosis	Preclinical study	[234]
Bromelain	Hepatic fibrosis	MDA↓, NO↓, GSH↑, SOD↑, CAT↑, GSH-Px↑	Suppresses ROS overexpression, fibrotic, and HSC activation to prevent hepatic fibrosis	Preclinical study	[235]
D-Carvone	Hepatic fibrosis	MDA↓, SOD↑, GSH↑, TAC↑	Inhibits TGF-β1/SMAD3 pathway to alleviate oxidative stress and inflammation in liver	Preclinical study	[236]
Notoginsenoside R1	Hepatic fibrosis	MDA↓, GSH↑, SDO↑, GST↑	Suppresses the activation of HSCs and exerts anti-oxidant and anti-inflammatory by inhibiting NF-κB and MAPK pathways	Preclinical study	[237]
Kinsenoside	Hepatic fibrosis	NO↓	Inhibits TGFβ1/SMAD/CTGF pathway and PI3K-AKT-FoxO1-mediated immune cell maturation and HSCs activation	Preclinical study	[238, 239]
Geniposide	Hepatic fibrosis	MDA↓, SOD↑, GSH-Px↑	Regulates AMPK/SITR1 pathways and Nrf2 signaling cascades in HSCs	Preclinical study	[240]
Astaxanthin	HCC	MDA↓, GSH↑	Inhibits proliferation and induces apoptosis of HCC cells; attenuates DNA damage and cell death	Preclinical study	[241]
Tiopronin	HCC	MDA↓, NO↓, CAT↑, GPx↑	Improves liver injury and abates hepatocarcinogenesis	Prospective study	[242]

CAT, catalase; CTGF, connective tissue growth factor; DAAs, direct-acting antivirals; DNA, deoxyribonucleic acid; ER, endoplasmic reticulum; GSH, glutathione; GSH-Px, glutathione peroxidase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSCs, hepatic stellate cells; IFN-α, interferon alpha; JNK, c-Jun N-terminal kinase; MDA, malondialdehyde; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; NO, nitric oxide; NOX4, NADPH oxidase 4; PTEN, phosphatase and tensin homolog; PI3K, phosphatidylinositol-3-kinase; ROS, reactive oxygen species; SIRT1, sirtuin 1; SMDA3, small mother against decapentaplegic 3; SOD, superoxide dismutase; STAT-1, signal transducer and activator of transcription 1; TAC, total antioxidant capacity; TGF-β1, transforming growth factor beta 1; TNF-α, tumor necrosis factor alpha

patients with advanced HCC. Similarly, in a multicenter phase II study with dose adjustment according to baseline serum bilirubin level, the antitumor activity of single agent irinotecan was not significant in advanced HCC, although it exhibited a favorable toxicity profile [222]. The above evidence suggests that antioxidant therapy may not show a good effect in advanced HCC with severe abnormal liver function. Therefore, antioxidant intervention is more essential in the early stages of HCC.

Increasing ROS to promote tumor cell death in the advanced stages of HCC

Certain agents specifically increase the generation of ROS or inhibit the activity of antioxidant system in tumor cells, thereby exerting a killing effect on HCC. Therefore, they have potential as HCC therapeutic drugs. Tables 2 and 3 summarize drug molecules that increase ROS, including natural potential products and clinically used drugs. Drug-induced tumor cell death due to excessive ROS production encompasses several distinct forms, such as apoptosis, autophagy, ferroptosis, pyroptosis, and necrosis.

Numerous clinical treatment drugs for HCC, including bevacizumab, sorafenib, lenvatinib, regorafenib, and cisplatin, increase ROS levels, activating the intrinsic apoptotic pathway [243–247]. It results in increased expression of cytochrome c, which forms apoptosomes with Apaf-1 and pro-caspase-9, subsequently reducing the levels of the anti-apoptotic protein Bcl-2 and promoting tumor cell apoptosis [248]. As the important first-line targeted agents for the treatment of HCC, sorafenib and lenvatinib also induce HCC death through producing ROS. Through the analysis of genome-wide clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) knockout, *Keap1* was identified as a sensitivity gene in HCC patients treated with sorafenib, lenvatinib, and regorafenib [249]. The deregulation of the Keap1/Nrf2 pathway following Keap1 inactivation contributes to resistance to sorafenib, lenvatinib, and regorafenib in human HCC cells through decreasing ROS levels. In HCC patients, serum levels of AOPP were higher when sorafenib treated ($P < 0.001$). An increase in

Table 2 Clinically used drugs that promote ROS in the advanced stages of HCC

Agent	Cell lines	Index changes	Effects	Clinical trial phases	Refs.
Bevacizumab	Hep3B and SMMC-7721	ROS↑	Inhibits tumor cells growth, promotes cell apoptosis, and increases DNA oxidative damage	Phase II trial	[243]
Sorafenib	Huh7, SMMC-7721, HepG2, Hep3B, SK-Hep-1, Hepa1–6 and H22	ROS↑ GSH↓	Damages mitochondrial morphology, reduces ATP synthesis and cell death in tumor cells	Phase III trial	[245]
Regorafenib	L02, Huh-7 and SMMC-7721	ROS↑	Induces mitochondrial dysfunction and cell death in tumor cells	Phase III trial	[246]
Doxorubicin	H22	ROS↑	Inhibits cancer cell viability, migration, and invasion	Phase II trial	[266]
Oxaliplatin	Huh7 and SMMC-7721	ROS↑	Increases tumor cell apoptosis	Phase III trial	[267]
Lenvatinib	Hep3B and Huh7	ROS↑, GSH↓	Induces ferroptosis and apoptosis by inhibiting FGFR4	Phase III trial	[261]
5-fluorouracil	Hepa1-6	ROS↑	Induces mitochondrial dysfunction and tumor cell death	Phase III trial	[268, 269]
Cisplatin	HepG2 and SMMC-7721	ROS↑	Induces the senescence of liver cancer cells	Phase III trial	[247]

ATP, adenosine triphosphate; FGFR4, fibroblast growth factor receptor 4; GSH, glutathione; ROS, reactive oxygen species

Table 3 Potential drugs that promote ROS in the advanced stages of HCC

Agent	Cell lines	Index changes	Effects	Phase	Refs.
1,8-Cineole	HepG2	ROS↑	Induces the senescence of liver cancer cells	Preclinical study	[270]
Thymoquinone	HepG2	ROS↑	Induces apoptosis in hepatoma cells	Preclinical study	[251]
Curcumin derivative	Sk-Hep-1 and HepG2	ROS↑	Induces apoptosis in hepatoma cells	Preclinical study	[254]
Koumine	Huh-7 and SNU-449	ROS↑	Decreases mitochondrial membrane potential and increases ROS production to induce apoptosis in hepatoma cells	Preclinical study	[271]
Kaempferol	HepG2	ROS↑	Promotes ROS and induces apoptosis	Preclinical study	[272]
Ginsenoside	HepG2 and LM3	ROS↑, GSH↓	Inhibits glutamine metabolism; induces cell cycle arrest and apoptosis	Preclinical study	[252]
Oleanolic acid	HepG2	ROS↑	Induces the apoptosis through downregulating PI3K/AKT/mTOR pathway, upregulating p53 pathway, and inducing oxidative stress	Preclinical study	[273]
Ellagic acid	HepG2 and Huh7	ROS↑, MDA↑, GSH↓	Enhances oxidative stress and induces apoptosis of cancer cells	Preclinical study	[274]
Fluoxetine	SK-Hep1 and Hep3B	ROS↑	Induces cell apoptosis through exogenous/endogenous pathways and inhibits anti-apoptotic and metastatic activity	Preclinical study	[275]
Silymarin	HepG2	ROS↑, GSH↓	Promotes tumor cell apoptosis by inhibiting Notch signaling	Preclinical study	[276]
MSDF	HA22T HA59T	ROS↑	Induces cancer cell apoptosis and autophagy	Preclinical study	[256]
Sanguinarine	Bel-7402 and Bel-7404	ROS↑	Induces ROS-dependent mitochondrial autophagy and apoptosis	Preclinical study	[189]
Coptisine	Hep3B	ROS↑	Induces mitochondrial dysfunction and autophagy	Preclinical study	[257]
Allicin	HepG2	ROS↑	Induces human liver cancer cell death through autophagy or apoptosis	Preclinical study	[258]
β-Thujaplicin	HepG2	ROS↑	Induces autophagic cell death, apoptosis, and cell cycle arrest	Preclinical study	[259]

Table 3 (continued)

Agent	Cell lines	Index changes	Effects	Phase	Refs.
Metformin	Huh7 and Hep3B	ROS↑	Increases lipid peroxidation levels, promotes ferroptosis, and increases sensitivity of Huh7 cells to sorafenib	Preclinical study	[277]
Polyphyllin I (PPI)	HepG2 and MHCC97H	ROS↑	Induces ferroptosis by inhibiting the Nrf2/HO-1/GPX4 axis	Preclinical study	[263]
Scutellaria barbata	SMMC-7721, HepG2 and Huh7	ROS↑	Induces ferroptosis of tumor cells through iron peroxidation and lipid peroxidation	Preclinical study	[262]
Mallotucin D	HepG2	ROS↑	Induces ROS-dependent mitochondrial autophagy and cell pyroptosis	Preclinical study	[278]
Miltirone	HepG2 and Hepa1-6	ROS↑	Inhibits tumor cell proliferation and induces tumor cell pyroptosis	Preclinical study	[123]
Neobavaisoflavone	HepG-2 and HCCLM3	ROS↑	Induces pyroptosis of liver cancer cells	Preclinical study	[264]
Apigetrin	Hep3B	ROS↑	Induces ROS-dependent apoptosis and necroptosis	Preclinical study	[125]
Arsenic trioxide (ATO)	H22 and Huh7	ROS↑	Induces ROS-dependent apoptosis and necroptosis	Preclinical study	[265]

AKT, Akt protein kinase; GSH, glutathione; GPX4, glutathione peroxidase 4; HO-1, heme oxygenase 1; MDA, malondialdehyde; mTOR, mammalian target of rapamycin; Nrf2, nuclear factor erythroid 2-related factor 2; Notch, notch signaling pathway; p53, tumor protein p53; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; ROS, reactive oxygen species

serum AOPP concentration ≥ 0.2 μmol/L chloramine T equivalent after 15 days of treatment is a predictive factor for sorafenib response with higher progression-free survival ($P < 0.05$) and overall survival rates ($P < 0.05$). It indicates that elevated serum AOPP levels in sorafenib-treated HCC patients correlate with the drug's clinical effectiveness and may serve as a potential biomarker of treatment response [250].

In addition to conventional chemotherapeutic and targeted agents, an increasing number of natural compounds have also demonstrated an ability to modulate ROS levels and trigger apoptosis in HCC cells, offering promising complementary therapeutic options. Thymoquinone, a natural product, upregulates ROS and oxidative stress-related genes *NQO1* and heme oxygenase-1 (*HO-1*), activating exogenous apoptotic pathways. Specifically, it enhances tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced HepG2 cell death by upregulating TRAIL death receptors, inhibiting NF-κB and IL-8, and stimulating apoptosis [251]. Ginsenoside Rk1 downregulates glutaminase 1 (GLS1) expression, reducing GSH production and stimulating ROS accumulation, thereby inducing cell apoptosis. Mechanistically, Rk1 induces apoptosis both in vitro and in vivo by modulating the ERK/c-Myc signaling pathway [252]. Additionally, various

natural products such as curcumin derivatives, kaempferol, and oleanolic acid increase ROS levels, further activate caspase-dependent apoptosis, and induce tumor cell death [253, 254].

Many drugs induce autophagic cell death in HCC cells by elevating ROS production. OSU-03012, a celecoxib derivative, inhibits the growth of the human HCC cell line Huh7 by increasing ROS accumulation and inducing autophagic cell death, and it also suppresses the growth of Huh7 tumor xenografts [255]. Similarly, the novel fluorene derivative 9-methanesulfonylmethylene-2,3-dimethoxy-9*H*-fluorene (MSDF) enhances ROS production, resulting in the formation of acidic vesicular organelles and elevated LC-3-II levels, which lead to autophagic cell death [256]. Several natural compounds, including sanguinarine, coptisine, allicin, and β-thujaplicin, also increase ROS production, induce HCC cells autophagy and death, and exhibit anticancer effects [189, 257–259]. These findings indicate that drug-mediated ROS induce HCC cell death by modulating autophagy-related signaling pathways.

In addition, some drugs promote other forms of liver cancer cell death by increasing ROS levels, including ferroptosis, pyroptosis, and necroptosis. Many clinical drugs for HCC, such as sorafenib and levatinib, are ferroptosis inducers that increase lipid ROS production and disrupt the occurrence of HCC [260, 261]. Furthermore, *Scutellaria barbata* and polyphyllin I (PPI) induce ROS-dependent ferroptosis to inhibit the proliferation, invasion, and metastasis of HCC cells [262, 263]. Moreover, miltirone and neobavaisoflavone (NBIF) significantly induce ROS accumulation in HepG2 and Hepa1-6 cells by inhibiting the ERK pathway activated by mitogen, ultimately leading to pyroptosis involving the GSDMD pathway [123, 264]. Certain drugs induce multiple forms of tumor cell death, including necrosis. Apigenin, a bioactive dietary flavonoid, induces ROS-dependent necroptosis and apoptosis in Hep3B cells. This is evidenced by a significant increase in necrotic apoptosis markers RIP3, p-RIP3, and p-MLKL, a reduction in the expression of the anti-apoptotic marker B-cell CLL/lymphoma extra-large (Bcl-xl), and an increase in the pro-apoptotic marker Bcl-2-associated X protein (Bax) [125]. Similarly, arsenic trioxide (ATO) treatment leads to the accumulation of ROS and the activation of multiple cell death pathways, including necroptosis and ferroptosis, in ATO-sensitive HCC cells. Furthermore, ATO has been shown to inhibit the growth of both subcutaneous and in situ liver tumors [265].

Notably, various drugs exert distinct regulatory effects on ROS at different stages of progression from chronic viral hepatitis to HCC, contributing to their therapeutic roles. The classic antidepressant fluoxetine inhibits HCV infection, reduces ROS production and lipid accumulation in Huh7.5 cells, and activates the host antiviral JNK/STAT-1 and peroxisome proliferator-activated receptor (PPAR) signaling pathways, thereby slowing the progression of chronic hepatitis. In different liver cancer cell lines, fluoxetine increases intracellular ROS levels and activates both intrinsic and extrinsic apoptotic pathways, promoting tumor cell apoptosis [225, 275]. Similarly, metformin alleviates oxidative stress in patients infected with HCV and mitigates liver damage. Conversely, in liver cancer cell lines Huh7 and Hep3B, as well as in mice with subcutaneous tumor grafts, metformin increases ROS and lipid peroxidation levels, induces ferroptosis in liver cancer cells, and enhances their sensitivity to sorafenib [223, 277]. Furthermore, silymarin effectively reduces antioxidant marker levels in the liver of patients with

hepatitis C. However, in the context of liver cancer, it increases intracellular ROS levels, inhibits the Notch1 signaling pathway, and suppresses tumor cell proliferation and migration, thereby promoting apoptosis [216, 276].

Taken together, ROS-clearing drugs may be beneficial for the prevention and early treatment of viral hepatitis-induced HCC. For middle and late stages of viral hepatitis-induced HCC, ROS-promoting drugs are more suitable.

Immunotherapy

Immunotherapies targeting ROS regulation are essential for halting disease progression during the malignant transformation from viral hepatitis to HCC, offering effective treatment benefits.

Immune checkpoint inhibitors (ICIs)

Growing evidence highlights the critical role of ROS in regulating the effectiveness of ICI therapy, offering new perspectives for combating the malignant transformation of viral hepatitis into HCC. Recent studies have shown that the pleiotropic cytokine IFN-γ, abundant in the HCC microenvironment, not only enhances ROS and lipid peroxide accumulation in liver cancer stem cells (LCSCs) but also promotes PD-L1 expression and facilitates its mitochondrial translocation, which activates Drp1-dependent mitochondrial fission and reshapes cancer cell metabolism by enhancing glycolysis. Targeting this pathway, genetic silencing of PD-L1 has been shown to restore sorafenib-induced ferroptosis, inhibit glycolytic reprogramming, and reduce the stemness properties of LCSCs, providing a solid theoretical basis for PD-L1-targeted strategies and combination ICI therapy in HCC [279, 280]. Additionally, ROS has been successfully leveraged in nanomedicine-based immunotherapy designs. A PD-L1-conjugated nanoliposome system co-delivering paclitaxel (PTX) and the P-glycoprotein inhibitor zosuquidar (ZSQ) enables pH-responsive drug release while elevating intracellular ROS levels to induce apoptosis. This strategy not only enhances antitumor efficacy in xenograft models but also reduces off-target liver toxicity [281]. Another promising approach uses a biologically responsive Au–miR-183 inhibitor nanocomposite, designed to target LCSCs. This system promotes ROS accumulation by depleting NADPH and hydrogen peroxide, triggering immunogenic cell death (ICD) and reshaping the tumor immune niche. As a result, dendritic cell maturation and $CD8^+$ T cell infiltration are enhanced, effectively transforming immunologically "cold" tumors into "hot" ones, and improving the therapeutic response to PD-L1 blockade [280].

Furthermore, advanced drug delivery systems such as iron-based micelles, phototriggered disassembly nanoplatforms, and nanoprecipitation-based constructs including IR-780@FOM-cRGD nanoparticles, TB/PTX@RTK micelles, and NanoFlox/NanoF-dUMP formulations have shown significant efficacy in remodeling the tumor microenvironment. These platforms regulate ROS generation to trigger ICD and ferroptosis, promote immune cell infiltration, and enhance antitumor immune responses, particularly when combined with PD-L1-targeted antibody therapies [268].

In summary, modulating ROS levels and their downstream pathways offers a powerful strategy to overcome cancer stemness and drug resistance in HCC. When combined with PD-L1-targeted interventions, these ROS-based approaches significantly enhance

immune activation and antitumor efficacy, highlighting their potential for improving treatment outcomes in virus-related HCC.

T cell therapy

ROS play a key role in regulating immune-mediated cell death, inducing ferroptosis, and enhancing the antitumor response in T cell-based therapies, offering a promising strategy for the treatment of virus-related HCC through multiple mechanisms.

Studies have shown that dihydroartemisinin (DHA), a derivative of artemisinin, inhibits cyclin-dependent kinases (CDKs) in HCC cells, leading to excessive ROS accumulation. During this process, DHA reduces the suppressive activity of immunosuppressive myeloid-derived cells, thereby enhancing the efficacy of immune checkpoint inhibitors such as anti-PD-1 antibodies and chimeric antigen receptor (CAR)-T cell therapies [282]. Further research has demonstrated that IFN-κ can synergistically sensitize tumor cells to ferroptosis inducers, through the IFNAR/STAT1/acyl-coA synthetase long-chain family member 4 (ACSL4) signaling axis, particularly in the presence of arachidonic acid. Moreover, CAR-T cells engineered to express IFN-κ have shown superior cytotoxicity against both antigen-positive and antigen-negative tumor cells in vitro, as well as enhanced anti-HCC effects in vivo [283].

In addition, T cell receptor (TCR)-engineered T cell therapy has emerged as a promising immunotherapy for HCC, although immune evasion by tumor cells remains a major challenge. Recent studies indicate that the combination of atorvaquinone (ATO), a compound known to elevate intracellular ROS, with TCR-T cell therapy can significantly increase ROS levels within both the cytoplasm and mitochondria, expand the labile iron pool, and destabilize mitochondrial membrane integrity in tumor cells, ultimately inducing ferroptosis. Transcriptome analysis further confirmed that this combination activates ferroptosis-related pathways, boosts IFN-γ secretion by TCR-T cells, and effectively suppresses tumor growth in HCC xenograft models without causing significant adverse effects [284]. In summary, the combined regulation of ROS and ferroptosis not only reshapes the immunosuppressive tumor microenvironment but also significantly enhances the efficacy of T cell-based immunotherapies in HCC.

Viruses

Virus-mediated multitarget therapeutic strategies have opened new avenues for treating the malignant transformation of viral hepatitis into HCC, with ROS emerging as a key regulatory factor in this process. One such approach involves a recombinant adenovirus engineered to co-express apoptin and melittin (MEL). This virus induces elevated ROS levels in HCC cells, leading to the upregulation of pro-apoptotic proteins, including Bax, cleaved caspase-3, and caspase-9, while simultaneously downregulating the anti-apoptotic protein Bcl-2. As a result, the recombinant virus effectively inhibits tumor cell proliferation, migration, and invasion, and demonstrates significant anti-tumor activity in subcutaneous HCC mouse models [285]. Another promising strategy utilizes an oncolytic cowpox virus (oncoVV) carrying the AVL homolog B (AVL), which reprograms liver cancer cell metabolism to enhance intracellular ROS production. The increase in ROS not only promotes tumor cell apoptosis but also facilitates viral replication, highlighting a self-amplifying therapeutic mechanism [286]. Additionally, the modification

of oncolytic adenoviruses with protein liposomes (PL) containing apolipoprotein A-I (ApoA-I) has been shown to significantly enhance viral gene delivery efficiency and stability, in both WT and V156K mutant forms. In zebrafish and nude mouse models of Hep3B tumor transplantation, PL-modified oncolytic viruses not only modulated ROS levels in vivo but also reduced tumor burden more effectively than unmodified viruses, improving the overall physiological state of the treated animals [287]. In summary, the regulation of ROS signaling pathways plays a central role in enhancing the antitumor activity of virus-based therapies.

Although immunotherapy strategies that modulate ROS levels have shown considerable promise, patient responses remain highly variable, largely owing to individual differences in tumor genetics and immune microenvironment profiles.

In proliferative HCC subtypes, often referred to as immune hot tumors, including TGF-β/Wnt-driven tumors and some HBV-related HCC such as aristolochic acid (AA)-associated mutation subtypes, the tumor microenvironment is typically characterized by abundant $CD8^+$ T cell infiltration and high PD-L1 expression [288]. However, these T cells are frequently in a state of functional exhaustion, as indicated by the upregulation of inhibitory receptors such as PD-1 and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) [289]. In this context, the production of ROS is generally active but maintained at a moderate level, a range often referred to as the "pro-inflammatory threshold." At this concentration, ROS is sufficient to enhance immune cell activation without directly triggering apoptosis. This moderate level of ROS plays a dual role: on the one hand, it activates the NF-κB pathway in dendritic cells, promoting the expression of major histocompatibility complex (MHC) and co-stimulatory molecules, which enhances antigen presentation efficiency; on the other hand, it modulates TCR signaling in $CD8^+$ T cells, thereby strengthening their proliferation and cytotoxic function [290, 291]. As a result, immune hot tumors tend to respond favorably to ICIs, such as anti-PD-1 antibodies. ROS and ICIs often act synergistically in these tumors, facilitating T cell activation, boosting IL-2 and IFN-γ secretion, and enhancing overall therapeutic efficacy [292].

In contrast, nonproliferative HCC subtypes, including CTNNB1-mutated tumors and HCV-related HCC, are often described as immune cold tumors. These tumors display sparse T cell infiltration and low antigen presentation capacity, and are dominated by myeloid-derived suppressor cells (MDSCs) and Tregs. Dense extracellular matrix barriers further limit immune cell access [289]. In these contexts, high ROS levels can suppress T cell function by oxidizing thiol groups on TCR signaling molecules. ROS also promotes the recruitment and polarization of MDSCs and tumor-associated macrophages (TAMs), which secrete IL-10 and TGF-β, reinforcing an immunosuppressive microenvironment and reducing ICI efficacy [293]. Additionally, antioxidant systems such as GPx and peroxiredoxins are often downregulated in cold tumors, impairing ROS clearance and exacerbating immune dysfunction [293]. For tumors with excessive ROS accumulation, local or systemic antioxidant therapies, including *N*-acetylcysteine or lipoic acid derivatives, can help restore ROS to a tolerable range, protect T cell function, and improve response to ICIs, while also reducing immune-related adverse events [294, 295].

In summary, the therapeutic efficacy of ROS-modulating immunotherapies in HCC is highly dependent on tumor subtype, genetic background, and the immune microenvironment, highlighting the need for personalized strategies that balance ROS regulation and immune activation to optimize treatment outcomes.

Innovative treatments

In recent years, a variety of innovative therapies based on ROS responses have emerged, offering new strategies to prevent and treat the malignant transformation from viral hepatitis to HCC.

One promising approach involves a composite delivery system combining thermosensitive hydrogel with ROS-responsive nanogels, designed for the synchronized release of regorafenib (REG) and the TGF-β inhibitor LY3200882 (LY). Upon in situ administration, REG is first released to inhibit tumor growth and induce ROS production. The elevated ROS then triggers the controlled release of LY, which blocks TGF-β–driven EMT and immune escape [296]. Meanwhile, multifunctional nanomedicine platforms designed to induce ferroptosis are also showing therapeutic promise. For instance, DHA loaded onto Fe^{3+}-doped MnO_2 nanosheets exploits the tumor microenvironment to trigger a cascade of oxidative damage. Upon GSH-mediated degradation, the system releases Fe^{2+}, Mn^{2+}, and DHA, which together generate a surge of ROS via Fenton and related reactions. The breakage of DHA's peroxide bridge further amplifies oxidative stress, while GSH depletion leads to GPX4 inactivation, lipid peroxide accumulation, and the induction of ferroptosis and apoptosis in liver cancer cells [297].

Photodynamics and sonodynamics are usually integrated with nanodelivery to enhance HCC treatment efficacy by promoting ROS and enable precision medicine. Through a self-assembly process, triptolide (TPL, a naturally derived anticancer agent) and chlorin e6 (Ce6, a photosensitizer) were co-loaded into pH/ROS dual-responsive mPEG-TK-PBAE nanoparticles. Under laser irradiation, Ce6 was released and generated abundant ROS, further accelerated the degradation of the nanosystem, significantly increased photodynamic therapy-induced oxidative stress, and augmented TPL-induced apoptosis in HepG2 cells, leading to synergistic anticancer effects in vitro. The potent anti-HCC ability and low systemic toxicity of TPL/Ce6 NPs were also proved in H22 tumor-bearing mice [298]. Compared with photodynamic therapy, ultrasound exhibits a deeper tissue penetration ability. Ultrasound-triggered ROS lenvatinib nanoparticles (Len-RNPs) were designed for targeted drug delivery in HCC. To be specific, ultrasound exhibited superior tissue penetration and can produce ROS at the tumor site to treat deeper tumors, and Len is an emerging molecular targeted agent for HCC. This novel and precise strategy combining Len and ultrasound therapy mitigated the toxicity of Len and effectively triggered a robust systemic antitumor immune response through the promotion of ROS [299].

Besides, a CRISPR/Cas9 delivery system (HMME@Lip-Cas9) controlled by ultrasound was also developed to target nuclear factor erythroid 2-like 2 (NFE2L2), a key regulator of oxidative stress defenses in cancer cells. This system employs hematoporphyrin monomethyl ether to generate ROS upon ultrasound irradiation, causing oxidative stress and lysosomal rupture. The lysosomal damage releases Cas9 ribonucleoprotein (RNP) complexes, which knock out NFE2L2, further compromising

the oxidative stress defense mechanisms in cancer cells. This combined approach enhances tumor cell apoptosis while mitigating the adverse effects typically associated with ROS-targeting therapies [300].

Despite the advancements in HCC treatment, clinical translation of these delivery systems still encounters obstacles [301]. One of the foremost obstacles is achieving precise tumor targeting. The therapeutic efficacy of these strategies largely depends on the selective accumulation of drug-loaded systems within the tumor microenvironment, a process often hindered by factors such as tumor heterogeneity, abnormal vasculature, and the dense extracellular matrix [302]. For example, the composite delivery system that combines thermosensitive hydrogels with ROS-responsive nanogels offers a promising strategy for synchronized drug release; however, its success relies heavily on accurate localization at the tumor site. Limited drug penetration and nonspecific distribution not only diminish the therapeutic effectiveness but also increase the risk of off-target effects. Similarly, in ultrasound-triggered ROS generation systems such as lenvatinib nanoparticles (Len-RNPs), precise tumor targeting is essential to ensure that ROS are generated exclusively at the tumor site, thereby minimizing damage to surrounding healthy tissues. In addition to targeting challenges, systemic toxicity remains a significant concern. Although many of these innovative treatments are designed to mitigate side effects, ROS-based therapies can still cause unintended oxidative injury to normal tissues [303]. For instance, photodynamic and sonodynamic therapies rely on ROS generation, and if the ROS are not strictly confined within the tumor region, healthy cells may also suffer oxidative damage. Likewise, multifunctional nanomedicine platforms designed to induce ferroptosis, such as DHA-loaded Fe^{3+}-doped MnO_2 nanosheets, may trigger excessive ROS generation and glutathione depletion beyond the tumor boundary, potentially leading to systemic inflammation, immune dysfunction, and off-target toxicity that could compromise patient safety. Moreover, while preclinical studies have demonstrated promising anticancer effects, the clinical efficacy of these ROS-based therapies remains to be fully validated. The lack of large-scale, well-controlled clinical trials continues to delay their clinical adoption. In particular, the long-term safety of approaches involving gene editing or high-level ROS induction is still uncertain. Issues such as off-target genetic modifications, immunogenic responses, and cumulative toxicity must be rigorously evaluated in clinical settings. For example, ultrasound-activated CRISPR/Cas9 delivery systems raise concerns about unintended gene editing and disruption of normal cellular processes, which could result in unpredictable biological outcomes. Cost and accessibility further complicate the clinical translation of these advanced therapies. The complexity of the delivery systems, including the need for high targeting precision, real-time imaging guidance, and multistep synthesis, significantly increases development and treatment costs. This economic barrier may limit the accessibility of these therapies, especially in resource-constrained healthcare systems, thereby widening the gap in treatment availability across different patient populations.

In light of these challenges, future research should prioritize comprehensive safety evaluations of these innovative delivery platforms and their combination strategies. Additionally, it is essential to investigate the durability of their therapeutic effects

and their ability to overcome resistance in HCC treatment. Such efforts will be key to facilitating the successful clinical translation of ROS-based therapies and unlocking their full potential in improving patient outcomes.

In summary, by integrating ROS-responsive nanomaterials with conventional drugs, photodynamic agents, sonodynamic strategies, and gene-editing tools, these approaches offer precise, spatiotemporal control over therapeutic delivery and enhance anti-HCC efficacy while minimizing systemic toxicity. However, despite encouraging preclinical results, challenges related to large-scale production, material stability, and long-term biosafety remain key barriers to clinical translation. Continued efforts to optimize these delivery systems and evaluate their therapeutic durability will be essential for advancing ROS-based strategies from bench to bedside.

Concluding remarks and future perspectives

In the context of HCC induced by chronic hepatitis, ROS, the natural byproducts of oxidative metabolism, play a dual role in disease progression, mediating both pro-tumorigenic and anti-tumorigenic effects. These opposing outcomes arise from the intricate regulatory influence of ROS on molecules, organelles, and cellular responses, all governed by complex signaling pathways within the dynamic microenvironment of liver tissue. At physiological levels, ROS serve as critical signaling molecules that regulate cellular homeostasis, proliferation, and repair mechanisms. However, dysregulated or excessive ROS production disrupts cellular redox balance, triggering oxidative stress and promoting deleterious processes, including DNA damage, protein oxidation, lipid peroxidation, and mitochondrial dysfunction. These molecular perturbations collectively contribute to chronic inflammation, genomic instability, and the malignant transformation of hepatocytes.

A novel aspect introduced in this review is the acknowledgment of the spatiotemporal heterogeneity of ROS, emphasizing their capacity to play distinct roles at various stages of disease progression, cellular fates, and on different target molecules, organelles, and cells. Temporal heterogeneity refers to the variation in ROS levels and their effects over the course of the disease—from the onset of hepatitis through liver fibrosis to HCC growth and metastasis. At early stages of the disease, low ROS levels support cell survival and proliferation by activating growth-promoting pathways, including MAPK, PI3K/AKT, and Nrf2 signaling. These pathways facilitate liver cell growth, angiogenesis, and tissue repair, which, under pathological conditions, can contribute to disease progression. Notably, the pro-survival effect of ROS in early HCC depends greatly on the tumor cell's mutational background. In tumors carrying TP53 mutations, the HCC-specific hotspot mutations R249S and R249M, the normal apoptotic response to oxidative stress may be compromised [304, 305]. Loss or alteration of functional p53 diminishes the cell's ability to initiate cell cycle arrest or apoptosis in response to ROS-induced DNA damage [306]. In contrast, in early HCC cases where the p53 pathway remains intact, excessive ROS levels are more likely to trigger apoptotic signaling, thereby preventing the survival and clonal expansion of damaged cells [307]. Similarly, mutations in components of the Wnt/β-catenin pathway can shift the cellular redox balance [308]. In such cases, moderate ROS may further amplify the pro-survival and proliferative signals already activated by these oncogenic mutations, providing a growth advantage. These observations

indicate that the impact of ROS on HCC cell fate is determined not only by their concentration or duration of exposure, but also by the specific mutational context that governs redox homeostasis and signal transduction. As the disease advances, chronic and excessive ROS levels accumulate, exacerbating liver damage and inducing a range of detrimental cellular fates. Specifically, elevated ROS levels lead to cell phenotype remodeling, whereby hepatocytes undergo transformation to more invasive or tumorigenic phenotypes, supported by metabolic reprogramming and epigenetic changes. Moreover, persistent oxidative stress induces cell senescence, a state of irreversible growth arrest associated with the secretion of pro-inflammatory factors that exacerbate the fibrotic and inflammatory environment. In some contexts, when ROS levels exceed cellular tolerance, they trigger cell death mechanisms, such as apoptosis or necrosis, contributing to liver tissue destruction and the establishment of a microenvironment conducive to HCC initiation. These temporally variable ROS effects underscore their dual role in promoting tumorigenesis at different stages of the disease.

The dual role of ROS is well established, yet whether this duality applies uniformly to specific stages of disease progression remains a subject of ongoing research and debate. Current evidence indicates that ROS can both promote and inhibit tumor growth, with the balance between these effects being highly context dependent. The cellular outcome of ROS exposure is influenced by dosage, duration, and the narrow concentration window in which ROS elicit beneficial signaling effects. Beyond this threshold, excess ROS typically induce harmful consequences. Moreover, HCC cells often adapt by upregulating antioxidant defenses, such as GSH and Nrf2 pathways, which can shift the toxicity threshold and modulate ROS effects. In addition, variations in the cellular environment, including cell type, microenvironment, and genetic background, further affect sensitivity to ROS-induced changes. Therefore, although ROS can promote cell survival and proliferation at lower concentrations and trigger cell death and genomic instability at higher levels, their biological effects are highly context dependent. Applying this dual-effect concept uniformly to specific disease stages, such as the transition from viral hepatitis to HCC, oversimplifies the complex interplay of intracellular signaling and microenvironmental factors, which can vary substantially between patients.

In parallel, spatial heterogeneity underscores the differential impact of ROS within specific cellular compartments, organelles, and biomolecular targets. For example, mitochondria serve as both a primary source and target of ROS, with mitochondrial dysfunction exacerbating ROS generation and creating a self-amplifying cycle of oxidative damage. This mitochondrial ROS overload impairs energy production and promotes the release of pro-apoptotic factors, directly influencing cell fate decisions such as apoptosis or necrosis. Similarly, ROS-induced stress at the ER disrupts protein folding and lipid metabolism, leading to ER stress and contributing to tumor progression by enhancing pro-survival pathways or triggering cell death under extreme oxidative conditions. These spatially distinct effects of ROS not only shape cell fate but also modulate the TME by influencing immune cell infiltration, angiogenesis, and extracellular matrix remodeling, all of which play critical roles in HCC progression.

Given the pivotal role of ROS in HCC progression, they have emerged as a promising focal point for treatment and intervention. Current strategies targeting ROS aim to balance oxidative stress by either safeguarding healthy cells through ROS suppression

or inducing apoptosis in tumor cells by elevating oxidative stress beyond tolerable levels [309, 310]. However, therapeutic applications of ROS modulation face challenges, particularly the systemic toxicity of ROS-targeting agents, which mirrors the substantial side effects associated with traditional chemotherapy and radiation therapy. These limitations necessitate the development of innovative therapeutic approaches that selectively exploit the heterogeneity of ROS in HCC.

Emerging therapies, including nanotechnology-driven delivery systems, TME-responsive platforms, and innovative modalities such as sonodynamic therapy (SDT), offer promising avenues for selectively targeting ROS in HCC. Moreover, integrating ROS modulation with other strategies, such as immune checkpoint inhibitors, gene-editing technologies, and combination therapies, holds significant potential to enhance treatment efficacy and reduce disease progression. However, the clinical translation of these approaches remains hindered by several challenges. ROS-based therapies such as photodynamic therapy, SDT, and nanomedicines can inflict off-target oxidative damage, while the absence of large-scale and well-controlled clinical trials further delays their development. To overcome these challenges, future research must prioritize comprehensive safety and biosafety evaluations, standardized large-scale manufacturing protocols, and cost-effective formulation strategies. Finally, deeper exploration of ROS heterogeneity and its dynamic interplay with the tumor microenvironment, coupled with rational combination approaches, will be instrumental in developing next-generation therapies to prevent and manage HCC.

In summary, this review emphasizes the critical importance of understanding the spatiotemporal heterogeneity of ROS in the progression of HCC induced by chronic hepatitis. By elucidating the dual role of ROS in promoting and inhibiting tumor development, this review provides a nuanced framework for interpreting their diverse impacts at cellular, organelle, and molecular levels. Such insights are essential for advancing precision medicine approaches that leverage ROS dynamics for therapeutic benefit.

Abbreviations

8-OHdG	8-Hydroxy-2′-deoxyguanosine
8-oxoGsn	8-Oxo-7,8-dihydroguanosine
AA	Aristolochic acid
ADORA1	Adenosine A1 receptor
AhR	Aromatic hydrocarbon receptor
AKT	Protein kinase B
AMPK	AMP-activated protein kinase
AOPP	Advanced oxidation protein products
Apaf-1	Apoptosis-inducing factor 1
ARE	Antioxidant response element
ASK1	Apoptosis signal-regulating kinase 1
ATF	Activating transcription factor
ATG	Autophagy-related gene
ATO	Arsenic trioxide
ATP	Adenosine triphosphate
Bax	Bcl-2-associated X protein
Bcl-2	B-cell CLL/lymphoma 2
BCR	B cell receptor
BER	Base excision repair
Bim	Bcl-2 interacting mediator of cell death
CAR	Chimeric antigen receptor
CAT	Catalase
CCL	C–C motif chemokine ligand
CDK	Cyclin-dependent kinase
CEBPs	CCAAT enhancer binding proteins

CIRP	Cold shock protein
COX	Cyclooxygenase
CTGF	Connective tissue growth factor
CTNNB1	Catenin beta 1
CSCs	Cancer stem cells
CYP450	Cytochrome P450
DAMPs	Damage-associated molecular patterns
DCs	Dendritic cells
DHA	Dihydroartemisinin
DHCR24	24-Dehydrocholesterol reductase
Drp1	Dynamin-related protein 1
ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
ETC	Electron transport chain
FADD	Fas-associated death domain protein
FGFR4	Fibroblast growth receptor 4
GLS1	Glutaminase 1
GPx	Glutathione peroxidases
GRP75	Glucose-regulated protein 75
GSH	Glutathione
GST	Glutathione *S*-transferase
GTP	Guanosine triphosphate
GSDMD	Gasdermin D
HBV	Hepatitis B virus
HBx	Hepatitis B virus X protein
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIF-1α	Hypoxia-inducible factor-1 alpha
HNE	4-Hydroxy-2-nonenal
HO-1	Heme oxygenase-1
HSCs	Hepatic stellate cells
HR	Hazard ratio
ICIs	Immune checkpoint inhibitors
ICD	Immunogenic cell death
IFN-γ	Interferon-gamma
IKK	IκB kinase
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IRE1	Inositol requiring enzyme 1
JAK2	Janus kinase 2
JNK	C-Jun N-terminal kinase
KCs	Kupffer cells
Keap1	Kelch-like ECH-associated protein 1
LC3-II	Microtubule-associated protein 1 light chain 3-II
LDL	Low-density lipoprotein
LPO	Lipid peroxidation
LPS	Lipopolysaccharide
MAM	Mitochondrial-associated endoplasmic reticulum
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
MDM2	Murine double minute 2
METTL3	Methyltransferase-like 3
MIF	Macrophage migration inhibitory factors
MLKL	Mixed lineage kinase domain-like pseudokinase
MMPs	Matrix metalloproteinases
MPTP	Mitochondrial permeability transition pore
mTOR	Mammalian target of rapamycin
NEIL1	Endonuclease VIII-like 1
NF-κB	Nuclear factor kappa-B
NFAT	Nuclear factor of activated T cells
NK cells	Natural killer cells
NLRP3	NLR family pyrin domain containing 3
NO	Nitric oxide
NOX1	NADPH oxidase 1
NQO1	NADPH quinone oxidoreductase 1
Nrf2	Nuclear factor erythroid 2-related factor
NS3/4A	Nonstructural protein 3/4A
NS5A	Nonstructural protein 5A

NS5B	Nonstructural protein 5B
NUPR1	Nuclear protein 1
OXPHOS	Oxidative phosphorylation
p53	Tumor protein p53
PDCD4	Programmed cell death 4
PD-L1	Programmed cell death-ligand 1
PI3K	Phosphatidylinositol-3-kinase
PIP3	Phosphatidylinositol 3,4,5-trisphosphate
PKG	Protein kinase G
PPAR	Peroxisome proliferator-activated receptor
PRDX4	Peroxiredoxin 4
PTEN	Phosphatase and tensin homolog
PTM	Post-translational modification
PTP1B	Protein tyrosine phosphatase 1B
PGE2	Prostaglandin E2
RIP3	Receptor interacting serine/threonine kinase 3
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RUNX3	Runt-related transcription factor 3
SAM	*S*-adenosylmethionine
SIRT	Sirtuin
SMAD	Mothers against decapentaplegic homolog
SOD	Superoxide dismutase
STAT3	Signal transducer and activator of transcription 3
TCR	T cell receptor
TGF-β1	Transforming growth factor beta 1
TME	Tumor microenvironment
TNF-α	Tumor necrosis factor-alpha
TLRs	Toll-like receptors
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
TrxR1	Thioredoxin reductase-1
Tregs	Regulatory T cells
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor
XO	Xanthine oxidase
XBP1	X-box binding protein 1
YTHDF1	YTH domain-containing family protein 1

Acknowledgements
We thank BioRender for providing drawing support. All images created with BioRender.com, with permission.

Author contributions
Huimin Yuan, Jia Liu, Ruochen Xu, Ruiyang Qu, Shuai Liu, and Keshan Yang wrote the review and drew the figures. Ming Xiang and Yonghui Zhang reviewed the manuscript and provided some helpful suggestions. The manuscript has been read and approved by all co-authors. All authors contributed to the review and approved the submitted version.

Funding
This work was supported by the National Natural Science Foundation of China (grant nos. 82071749 and 82371824).

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no competing of interest.

Received: 20 January 2025 Accepted: 20 May 2025
Published online: 14 June 2025

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