Immune and Metabolic Alterations in Liver Fibrosis: A Disruption of Oxygen Homeostasis?

Xinyu Li1,2†, Quyan Zhang1,2†, Zeyu Wang2, Quan Zhuang3* and Mingyi Zhao1*

1Department of Pediatrics, The Third Xiangya Hospital, Central South University, Changsha, China, 2Xiangya School of Medicine, Central South University, Changsha, China, 3Transplantation Center, The Third Xiangya Hospital, Central South University, Changsha, China

According to the WHO, “cirrhosis of the liver” was the 11th leading cause of death globally in 2019. Many kinds of liver diseases can develop into liver cirrhosis, and liver fibrosis is the main pathological presentation of different aetiologies, including toxic damage, viral infection, and metabolic and genetic diseases. It is characterized by excessive synthesis and decreased decomposition of extracellular matrix (ECM). Hepatocyte cell death, hepatic stellate cell (HSC) activation, and inflammation are crucial incidences of liver fibrosis. The process of fibrosis is also closely related to metabolic and immune disorders, which are usually induced by the destruction of oxygen homeostasis, including mitochondrial dysfunction, oxidative stress, and hypoxia pathway activation. Mitochondria are important organelles in energy generation and metabolism. Hypoxia-inducible factors (HIFs) are key factors activated when hypoxia occurs. Both are considered essential factors of liver fibrosis. In this review, the authors highlight the impact of oxygen imbalance on metabolism and immunity in liver fibrosis as well as potential novel targets for antifibrotic therapies.

Keywords: liver fibrosis, oxidative stress, hypoxia-inducible factor, immunometabolism, mitochondrial dysfunction

BACKGROUND

Liver cirrhosis is an irreversible liver fibrosis and was the 11th leading cause of death worldwide from 2000 to 2019 according to the WHO (WHO, 2020). Liver fibrosis is a common pathological pathway for various liver diseases, including viral hepatitis, toxic hepatitis, autoimmune hepatitis, and metabolic and genetic liver diseases (Iredale and Campana, 2017). It is a response to persistent liver injury and is characterized by excessive deposition of extracellular matrix (ECM) (Alegre et al., 2017). In the end stage, most liver fibrosis progresses into liver cirrhosis, with the loss of normal function. Early liver fibrosis is reversible, but once it develops into cirrhosis, it is irreversible and leads to functional failure and many complications, such as portal hypertension. Therefore, the prevention and reversal of fibrosis is the main aim for the treatment of liver diseases (Schuppan et al., 2018).

The death of hepatocytes, activated hepatic stellate cells (HSCs), and inflammation are the main causes of the development of liver fibrosis (Alegre et al., 2017). An imbalance in oxygen supply can lead to injury to organ parenchymal cells to secrete various fibrotic and inflammatory cytokine factors and recruit inflammatory cells to the injured stroma in chronic diseases (Darby and Hewitson, 2016; Liu et al., 2017). Hypoxia is rather common in chronic liver diseases. There are several pathways regulating oxygen homeostasis, and hypoxia-inducible factor-1α (HIF-1α) is one of them. HIF-1α, activated in hypoxia, could regulate the transcription of many genes (Kietzmann,
Mitochondrial dysfunction can be detected in many kinds of liver diseases and is believed to be involved in liver fibrosis (Mansouri et al., 2018). It is characterized by ultrastructural mitochondrial lesions, respiratory chain activity reduction, ATP depletion, excessive ROS levels, and mitochondrial DNA (mtDNA) damage. A recent article reported that mitochondrial dysfunction in peripheral cells along with alterations in metabolites of the urea cycle may act as biomarkers for the progression of fibrosis in nonalcoholic fatty liver disease (NAFLD) (Ajaz et al., 2021). The mitochondrial quality control system is crucial for the maintenance of mitochondria, including mitochondrial biogenesis, fusion and fission, and mitophagy.

Mitochondrial biogenesis is regulated by peroxisome proliferator-activated receptor-gamma (PPAR γ) coactivator-lalphap (PGC-1α) (Wu et al., 1999), which is a kind of transcriptional coactivator that regulates the expression of several transcription factors, such as nuclear respiratory factor (NRF)-1 and NRF-2 (Bhatti et al., 2017; Kiyama et al., 2018), and mitochondrial transcription factor A (TFAM) (Kang et al., 2007). A recent study proved that astaxanthin attenuated hepatocyte injury and the upregulation of PGC-1α (Zhai et al., 2015; Zhang et al., 2015; Tai et al., 2016; Raffaele et al., 2019; Raffaele et al., 2020). Mitochondrial fusion is regulated by the fusion proteins mitofusin 1 (Mfn1) and Mfn2 and optic atrophy 1 (OPA1), while mitochondrial fission in mammals is regulated by dynamin-related protein 1 (Drp1) (Ni et al., 2015). The role of PGC-1α in mitochondrial dynamics has been reported. In oxidative stress-induced mitochondrial damage, the downregulation of PGC-1α is related to abnormal mitochondrial fission. Hence, PGC-1α overexpression reduced Drp1 protein levels and prevented liver fibrosis (Zhang et al., 2020). Partially matter ≤2.5 μm (PM2.5) also contributes to mitochondrial dynamics dysfunction by increasing Drp1 and decreasing PGC-1α levels (Wang et al., 2021). The overexpression of HK domain-containing 1 (HKDC1) in the liver induced a defect in mitochondrial function by increasing Drp1 (Pusec et al., 2019). The antifibrotic effect of lipoic acid (LA) may be related to the upregulation of Drp1 (Luo and Shen, 2020).

Impaired Mitophagy and Liver Fibrosis
Mitophagy refers to the removal of dysfunctional mitochondria through fusion with lysosomes (Yoo and Jung, 2018). It can be classified into Pink1–Parkin-mediated mitophagy and Parkin-independent mitophagy. Mitophagy mediated by Drp-1 was activated by PM2.5, leading to HSC activation and fibrosis. Chronic CCl4 exposure impaired mitophagy in the liver, and melatonin may attenuate liver fibrosis by elevating PINK1 and Parkin (Kang et al., 2016). Researchers have shown that mitochondrial depolarization (mtDepo) occurs early in mice...
fed a Western diet (high fat, fructose, and cholesterol) and increases mitophagic burden. Together with suppressed mitochondrial biogenesis and mitochondrial depletion, mitochondrial damage promotes the progression of liver fibrosis (Krishnasamy et al., 2019). It is worth mentioning that Parkin-independent mediators, including Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) and NIX, can be regulated by HIF-1, thus removing damaged mitochondria and protecting against ROS accumulation.

**Impaired mtDNA Homeostasis and Liver Fibrosis**

In the case of mitochondrial dysfunction, mitochondrial DAMPs (mtDAMPs) are released to the extracellular space. This can stimulate liver inflammation and promote liver fibrosis. A recent study reported that mtDAMPs released from impaired hepatocyte mitochondria could directly activate HSCs (An et al., 2020). The role of mtDAMPs in liver fibrosis will be discussed in detail later.

**Reactive Oxygen Species Generation in Mitochondria**

The mitochondrial respiratory chain is considered the main source of ROS production. ROS refers to oxygen free radicals and nonradical oxidants (Zorov et al., 2014). It can be produced in both the mitochondrial matrix and the intermembrane space (Bouchez and Devin, 2019). Under normal homeostasis, mitochondria can remove physiological ROS by antioxidant mechanisms and metabolic adaptations (Lee et al., 2019) and thus maintain a balance between ROS production and removal. The antioxidant system includes superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and other antioxidants (Bhatti et al., 2017).

However, the excessive level of ROS caused by increased ROS production and decreased ROS scavenging may lead to protein, DNA, and lipid damage to mitochondria (Zorov et al., 2014; Sorrentino et al., 2018). Additionally, ROS can activate several pathways. Under hypoxia, with low-intensity ROS, the HIF-1α pathway is activated, leading to metabolic adaptation and subsequently inhibiting ROS production (Finkel, 2012). With moderate intensity of ROS in hypoxia, it can regulate inflammatory reactions, such as NACHT, LRR, and PYD domain-containing protein 3 (NLRP3) inflammasome, and mitogen-activated protein kinase (MAPK) signalling (Win et al., 2018). With a high intensity of ROS, apoptosis and autophagy may occur, preventing more damage.

Considering that changes in mitochondrial physiological processes participate in the development and progression of liver diseases, mitochondria are believed to be a promising treatment target for liver fibrosis.

**Role of the HIF Pathway in Liver Fibrosis**

Hypoxia and HIFs have been acknowledged as important drivers of liver fibrosis (Strowitzki et al., 2018). In fact, hypoxia is involved in a variety of liver diseases. Hypoxia levels are elevated in liver cancer and may be involved in destroying the natural immune response and creating an immunosuppressive microenvironment (Yuen and Wong, 2020). In NAFLD, hypoxia may mediate hepatic steatosis and abnormal lipid metabolism (Mesarwi et al., 2019). Hypoxia-mediated abnormal immune and metabolic microenvironments are also key factors in the development of fibrosis and liver cirrhosis.

The interaction of HIF-1α and Rho-associated coiled-coil-forming kinase 1 (ROCK1) promotes cell proliferation and collagen synthesis in rat HSCs under hypoxia (Hu et al., 2018). A recent study also showed that activated HIF-1α or HIF-2α in hepatocytes stimulates upregulation of chemokine ligand 12 (Cxc12) by converting latent transforming growth factor β (TGF-β) to active TGF-β (Strickland et al., 2020). Cxc12 is involved in the process of liver fibrosis through chemotaxis and activation of HSCs (Li et al., 2020). These findings indicate that HIF acts as an important regulator of liver fibrosis-targeting HSCs (Tirosh, 2018).

During liver fibrosis, hepatic sinusoidal blood flow disorder and hypoxia lead to the secretion of angiogenic factors by liver intrinsic cells. Pathological angiogenesis destroys the hepatic architecture and aggravates liver fibrosis (Poirier et al., 2017). Recent studies identified that hedgehog signalling promoted prospero homeobox protein 1 (PROX1) expression in liver fibrosis, which inhibited HIF-1α ubiquitination via a deubiquitinase called ubiquitin specific peptidase 19 (USP19). This hedgehog signalling-mediated hypoxia response participates in liver sinusoidal endothelial cell (LSEC) angiogenesis and the activation of HSCs (Feng et al., 2019; Yang et al., 2020). It has also been suggested that activated peroxisomal proliferator receptor γ (PPARγ) in HSCs could inhibit the expression of HIF-1α via an SMRT-dependent mechanism. The activation of PPARγ improved angiogenesis and vascular remodelling in carbon tetrachloride (CCL4)-induced liver fibrosis in rats. A possible negative feedback loop was raised in which HIF-1α may induce PPARγ expression in response to hypoxia or pathological stress, and then overexpressed PPARγ would inhibit HIF-1α transcription to limit amplification (Zhang et al., 2018).

**The Oxygen Imbalance Mediated-Immune and Metabolic Alterations in Liver Fibrosis**

**Mitochondrial Dysfunction Mediated-Immune Regulation in Liver Fibrosis**

Sterile inflammation (SI) is a common response to stress and injury in liver disease (Chen et al., 2018). This is a major process in the development of fibrosis and carcinogenesis (Kubes and Mehal, 2012). In SI, DAMPs, which are usually in the intracellular space, are released to the local microenvironment when infections, metabolic disorders, and other stimuli result in hepatocyte cell death, leading to a wide range of immune responses (Iredale and Campana, 2017). Several DAMPs have been identified, including mtDNA and nuclear DNA, high mobility group box-1 (HMGB-1), ATP and other molecules. HMGB-1 is released mostly by hepatocytes and Kupffer cells (KCs). Recent research confirmed the role of HMGB1 in liver fibrosis. It has been reported that HMGB1 could increase collagen...
type I production in HSCs via the receptor for advanced glycation end-products (RAGE), leading to liver fibrosis. The underlying mechanism was the pMEK1/2/pERK1/2/pJun signalling pathway (Ge et al., 2018). Furthermore, HMGB1 also links hepatocyte injury to hepatocellular carcinoma (HCC) (Hernandez et al., 2018).

MtDAMPs, including mtDNA and formyl peptides, are released to the extracellular space in the case of ROS-driven mitochondrial membrane permeability transition (Mihm, 2018). They recognize pattern recognition receptors (PRRs) on target cells to modulate the function of antigen-presenting cells (APCs), eosinophils, mast cells, and neutrophils (Krysko et al., 2011). For example, mtDNA recognizes Toll-like receptor 9 (TLR9) and NLRP3, and N-formyl peptides (NFPs) recognize TLR9 (Kubes et al., 2016). This revealed the potential of TLR9 ligand therapies. In patients with nonalcoholic steatohepatitis (NASH), mtDNA levels have been reported to increase (Garcia-Martinez et al., 2016). Furthermore, it increased in patients with active NASH (NAS 4–8 versus NAS 0–3, p = 0.0334) (An et al., 2020). It can activate several pathways (Zhang et al., 2019). The first is NLRP3 inflammasomes. NLRP3 can directly activate HSCs, triggering liver fibrosis (Inzagurat et al., 2019). Furthermore, it has been reported that in the mouse NASH model, mtDNA released by KCs bound and activated the NLRP3 inflammasome to induce interleukin (IL)-1β and IL-18, triggering proinflammatory responses and resulting in liver fibrosis (Shimada et al., 2012; Pan et al., 2018; Hu et al., 2019). Likewise, KCs induced by palmitic acid (PA) induced mtDNA and activated the NLRP3 inflammasome (Pan et al., 2018). The second is TLR9. In a mouse model of NASH, the plasma level of mtDNA increased, and it could activate TLR9, leading to a proinflammatory response (Garcia-Martinez et al., 2016). Therefore, the activation of TLR9 by mtDNA is believed to play a role in the transition from steatosis to steatohepatitis (Garcia-Martinez et al., 2016). The use of the TLR7/9 antagonist IRS954 could block the ability of mtDNA, resulting in a decrease in steatosis, ballooning and inflammation, serum transaminases, and inflammatory cytokine transcript levels (Garcia-Martinez et al., 2016). This revealed the potential of TLR9 ligand therapies. In acute liver injury induced by acetaminophen (APAP), mtDNA, which binds to TLR9, can induce neutrophil activation and liver inflammation. The crucial mediator is microRNA-223 (miR-223), which acts as a negative feedback loop to limit neutrophil overactivation and liver injury (He et al., 2017). DNA-sensing pathways could induce type I interferon (IFN-1) production in liver NPCs, which is related to hepatocyte necrosis (Araujo et al., 2018). The third is cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING). In a mouse model of NASH, STING deficiency attenuated steatosis, fibrosis, and inflammation in the liver; exposure to a STING agonist led to hepatic steatosis and inflammation in WT mice but not in STING-deficient mice (Yu et al., 2018). STING functions as a miRNA sensor in KCs and increases the expression of TNF-α and IL-6 through the nuclear factor-κB (NF-κB) signalling pathway in NASH (Yu et al., 2018). The activation of STING in macrophages is also related to liver fibrosis (Luo et al., 2018). Furthermore, other mediators related to mitochondria are formyl peptides. Formyl peptides are released from dysfunctional mitochondria and bind to formyl peptide receptors (FPRs) (Krysko et al., 2011). FPRs are expressed at high levels on neutrophil granulocytes and mononuclear phagocytes (Mihm, 2018). This binding leads to the migration of immune cells to the injured tissue, activating proinflammatory responses (Raoof et al., 2010). The role of formyl peptides has been revealed in systemic inflammatory response syndrome (SIRS) and ischaemia/reperfusion injury (IRI) (Zhang et al., 2010; Hu et al., 2015). However, the direct effect of formyl peptides on liver fibrosis has not been identified.

Moreover, mitochondria are the site of ATP production, and when in disease, high concentrations of ATP may be released extracellularly. ATP binds to P2X7 receptors on neutrophils, inducing NLRP3-ASC-caspase-1 inflammasome and IL-1β secretion (Schroder and Tschopp, 2010; Karmakar et al., 2016).

These studies revealed the crucial role of mtDAMPs in modulating the immune response and liver fibrosis, which are promising biomarkers and treatment targets.

**HIF Mediated-Metabolic Regulation in Liver Fibrosis**

An increasing number of studies have confirmed that HIF-1 acts as a crucial regulator in metabolic reprogramming in liver fibrosis (Corcoran and O’Neill, 2016). Glucose transporters 1 (GLUT1) and pyruvate kinase isozymes M2 (PKM2) are confirmed to be the target genes of HIF-1 and the key molecules of the Warburg effect (Wan et al., 2019). Wan et al. (2019) reported that HIF-1 upregulated GLUT1 and PKM2 expression in fibrotic liver and exosomes derived from activated HSCs. Interestingly, exosomes from activated HSCs were absorbed by KCs, LSECs, and quiescent HSCs, which enhanced glycolysis of these liver nonparenchymal cells. These findings represent a novel mechanism: upon injury of parenchymal hepatic cells, HIF-1 can regulate nonparenchymal cells (NPCs) to maintain synchronization of metabolic reprogramming.

In mice fed a high-fat diet (HFD), hepatic steatosis leads to liver tissue hypoxia. The HIF1-mediated PTEN/NF-kB-p65 pathway plays a critical role in the development of NAFLD to liver fibrosis (Han et al., 2019). In an apolipoprotein E-deficient (Apoe−/−) mouse model, the circadian locomotor output cycle kaput (CLOCK) protein indirectly regulates HIF1α expression by modulating PHD protein levels. In CLOCK deficiency, HIF1α binds to the Cd36 promoter, promoting CD36 expression and uptake of fatty acids in the liver. This regulatory link among hypoxia, metabolism, and circadian locomotor promotes cirrhosis in NAFLD (Pan et al., 2020). Studies of high cholesterol diet (HCD)-induced liver fibrosis revealed that inducible nitric oxide synthase (iNOS)-mediated enhancement of fibrosis was associated with HIF1α stabilization (Anavi et al., 2015). It has been suggested that cholesterol-mediated activation of HIF-1 is ROS- and nitric oxide (NO)-dependent. Cholesterol load increased mitochondrial dysfunction and NO levels, which promoted HIF-1 stabilization and transcriptional activity (Anavi et al., 2014). Succinate, an intermediate of the tricarboxylic acid...
cycle, accumulates in hepatocytes due to enhanced fatty acid oxidation in fibrosis. Accumulated succinate stabilizes and activates HIF-1α by impairing PHDs, which induces inflammation and HSC activation (Cho, 2018; She et al., 2018). We summarized the details of the mechanism above in Figure 1.

This evidence indicates that the oxygen balance in liver fibrosis is disrupted, which mediates metabolic disorders and the pathological accumulation of metabolic substances. There is no doubt that HIF-mediated oxygen balance control is a potential target for metabolic liver disease.

**HIF Mediated-Immune Regulation in Liver Fibrosis**

Although HIF-α is often activated in liver diseases, the roles of HIF-1 mediated-immune regulation in different liver injuries are different. In cholestatic liver disease, nuclear HIF-1α protein was
present in hepatocytes, liver macrophages, and liver fibroblasts of patients with primary biliary cirrhosis and primary sclerosing cholangitis (Copple et al., 2012). A study of cholestatic mice indicated that chronic liver injury activated HIF-1α in macrophages. Activated HIF in macrophages may regulate the expression of platelet-derived growth factor-B (PDGF-B) to promote fibrosis, which induces HSC proliferation, chemotaxis, and collagen production. In NASH mice, the significant upregulation of HIF-1α in hepatocytes increased proportion of M2 macrophages and promoted liver fibrosis and HCC (Ambade et al., 2016). Furthermore, HIF-1α is not only upregulated in hepatocytes, where it induces steatosis, but also in macrophages of NASH patients (Wang et al., 2019). The role of HIF-1α in macrophages in NASH was explored in the methionine-choline-deficient (MCD) diet-fed mice. Mice with stabilized HIF-1α levels in macrophages showed higher steatosis and liver inflammation. HIF-1α impaired autophagic flux in macrophages and upregulated NF-κB activation and monocyte chemoattractant protein-1 (MCP-1) production, leading to MCD diet-induced NASH (Wang et al., 2019). At the same time, digoxin was reported to be protective in NASH (Zhao et al., 2019). The role of HIF-1α in macrophages showed a higher steatosis and liver inflammation. HIF-1α impaired autophagic flux in macrophages and upregulated NF-κB activation and monocyte chemoattractant protein-1 (MCP-1) production, leading to MCD diet-induced NASH (Wang et al., 2019). The protective effect was related to the downregulation of HIF-1α transactivation (Ouyang et al., 2018).

A recent article has reported the role of HIF-1α in viral hepatitis type B that HIF-1α down-regulated apolipoprotein B mRNA editing enzyme catalytic subunit 3B (APOBEC3B) and thus impaired the anti-HBV effect of A3B (Riedl et al., 2021). However, HIF-1α also induced deoxyribonucleases (DNases), which limited the duplication of hepadnaviruses (Haller et al., 2019).

In contrast, the role of HIF-1α in chemicals-induced acute liver injury is different from its role in chronic liver diseases. Mochizuki et al. proposed a model in which liver necrotic cells could activate HIF-1α in HSCs through regional hypoxia or other mechanisms yet to be determined. HIF-1α then stimulated recruited macrophages to remove necrotic hepatocytes. In HSC-specific HIF-1α knockout, the levels of M1 macrophage activation markers and the percentage of Gr1hi macrophages in the liver were reduced, which impaired the clearance of necrotic cells and promoted fibrosis (Mochizuki et al., 2014). In APAP-induced liver injury, T-cell-specific Hif-1α gene knockout mice sustained severe liver damage, which was related to the aggravated inflammatory responses by enhancing aberrant innate-like γδ T-cell recruitment and excessive neutrophil infiltration (Suzuki et al., 2018).

It has also been reported that hepatocyte-specific deletion of HIF-2α improved NAFLD-associated fibrosis through downregulated histidine-rich glycoprotein (HRGP). The fraction of inflammatory Ly6C<sup>hi</sup> hepatic macrophages, its production of IL-12, and the expression of M1 cytokines/chemokines were significantly decreased in HIF-2α<sup>−/−</sup> mice. These findings indicated that HIF-2α/HRGP in parenchymal cells could promote proinflammatory responses of hepatic macrophages (Bartneck et al., 2016; Morello et al., 2018). These studies suggest that although liver injury is usually accompanied by the activation of HIF, different activated cells may have opposite effects on fibrosis. We summarized the oxygen imbalance mediated-immune alterations above in Figures 2A,C.

### THERAPEUTIC SIGNIFICANCE OF OXYGEN HOMEOSTASIS IN LIVER FIBROSIS

#### Therapies Targeting Mitochondrial Dysfunction to Alleviate Fibrosis

Since increasing evidence has proven the crucial role of mitochondria in liver fibrosis, several efforts have been made to assess the efficacy of pharmacologic therapies targeting mitochondria.

Attenuated mitochondrial dysfunction, increased mitochondrial fission, decreased HSC migration and activation, and decreased oxidative stress are involved in the protective role of augmenting liver regeneration (ALR) in liver fibrosis (Song et al., 2011; Ai et al., 2018). Ming Song et al. first reported the therapeutic effect of ALR gene therapy (Song et al., 2011). The underlying mechanisms were attenuating mitochondrial dysfunction and oxidative stress and inhibiting the activation of HSCs. The results of Ai et al. (2018) were consistent with the former results. The inhibition of ALR expression aggravated liver fibrosis in mice that were administered CCl<sub>4</sub> by promoting mitochondrial fusion and HSC migration. The inhibition of ALR may lead to increased mitochondrial Ca<sup>2+</sup> influx in HSCs, resulting in HSC migration. ALR transfection inhibited F-actin assembly, retarded HSC migration, and promoted mitochondrial fission (Ai et al., 2018).

Poly (ADP-ribose) polymerase (PARP) activation was found in patients with hepatic cirrhosis, and the inhibition of PARP had antifibrotic effects (Mukhopadhyay et al., 2017). PARP inhibition or genetic deletion of PARP1 was reported to attenuate alcohol-induced hepatic oxidative stress and mitochondrial dysfunction by improving the activity of complexes I and IV (Mukhopadhyay et al., 2017). Xing Lin et al. reported that didymin could alleviate liver injury and fibrosis induced by CCl<sub>4</sub> by inhibiting HSC proliferation and inducing apoptosis (Lin et al., 2016).

HSC apoptosis was partly mediated by MPTP opening. Didymin treatment led to cytochrome c release into the cytosol and decreased Bcl-2 expression, resulting in HSC apoptosis (Lin et al., 2016). Similarly, the curative effect of dihydroartemisinin (DHA) on liver fibrosis was also partly mediated by HSC apoptosis by releasing cytochrome c and activating the caspase pathway (Chen et al., 2016a).

Oxidative stress is a main stimulative factor of liver fibrosis, and it is a promising target. Melatonin may improve hepatic mitochondrial functions and thus reduce oxidative stress in some diseases (Coto-Montes et al., 2012; Jiménez-Aranda et al., 2014; Agil et al., 2015). In CCl<sub>4</sub>-induced liver fibrosis rats, melatonin protected against liver fibrosis by attenuating mitochondrial dysfunction, which was manifested by improved mitophagy and mitochondrial biogenesis (Kang et al., 2016). Melatonin also attenuated lipid-mediated mitochondrial dysfunction and ROS generation in hepatocytes and improved mitochondrial respiratory functions, leading to decreased oxidative stress and inflammation and thus inhibition of HSC activation (Das et al., 2017). Another mitochondria-targeted antioxidant, mitoquinone,
could attenuate liver fibrosis by reducing hepatic oxidative stress, preventing apoptosis, and promoting the removal of dysfunctional mitochondria (Turkseven et al., 2020).

It has been reported that adiponectin and its receptors enhanced fatty acid oxidation and glucose uptake and prevented the activation of HSCs induced by CCl4, thus alleviating NASH and fibrosis in mouse models (Xu et al., 2020).

Mitophagy is a selective form of autophagy that eliminates dysfunctional mitochondria (Williams et al., 2015a). It protects the liver from both acute and chronic ethanol consumption (Ma et al., 2020). Targeting mitophagy may protect the liver from acetaminophen and alcohol injury (Williams et al., 2015b). Interestingly, chronic deletion (KO) of Parkin alleviated APAP-induced liver injury, but acute knockdown of Parkin exacerbated injury (Williams et al., 2015a). This result suggested other protective pathways in the liver.

**Therapies Targeting the HIF Pathway to Alleviate Fibrosis**

Since hypoxia and HIFs are considered to be important drivers of liver fibrosis, targeting HIF may be an effective treatment for fibrosis (Strowitzki et al., 2018). In mice fed with HFD, curcumin can inhibit succinate-induced HSC activation by blocking the HIF-1α signalling pathway in mouse primary HSCs (She et al., 2018). In acute liver injury Tamoxifen, an agonist of the G protein-coupled oestrogen receptor (GPER), has been confirmed to inhibit the HIF1α pathway and prevent HSC activation by a mechanical mechanism (Cortes et al., 2019). In a rat model of CCl4-induced liver fibrosis, ligustrazine alleviated hepatic injury, angiogenesis, and vascular remodelling by decreasing the level of HIF-1α (Zhang et al., 2018). The combination of celecoxib and octreotide decreased thioacetamide-induced liver fibrosis in rats by inhibiting the phosphorylation of the extracellular signal-regulated kinase (p-ERK)/HIF-1α/vascular endothelial growth factor (VEGF) pathway (Gao et al., 2016). MicroRNA-122 can protect the liver from ethanol-induced injury and fibrosis by inhibiting HIF-1α expression (Satishchandran et al., 2018). In cholestatic liver fibrosis, it has also been reported that EW-7197, a TGF-β Type I receptor kinase inhibitor, can inhibit HIF1α-induced epithelial mesenchymal transition to alleviate cholestatic liver fibrosis (Kim et al., 2016). In NASH, isochlorogenic acid B was reported to have anti-fibrosis effects by inhibiting HSC activation,
attenuating oxidative stress via Nrf2, and suppressing multiple profibrogenic factors through miR-122/HIF-1α signalling pathway (Liu et al., 2019). The protective role of digoxin in steatohepatitis was related to the inhibition of PKM2/HIF-1α signalling pathway (Ouyang et al., 2018; Zhao et al., 2019).

MSCs Act as a Bridge to Link Immunometabolism, Oxygen Homeostasis, and Fibrosis

Mesenchymal stem cells (MSCs) are pluripotent stem cells that can be induced to differentiate into several tissue cells (Chen et al., 2016b). MSC sources are diverse, such as in bone marrow, adipose tissue, placenta, amniotic tissue, cord, lung, liver, and skin (Zhuang et al., 2019). Existing studies have shown that MSC therapy is prominently effective in hepatic fibrotic diseases indirectly by regulating the immune metabolism microenvironment (El Agha et al., 2017). The secretion of IL-17A from Th17 cells can promote fibrosis by activating fibroblasts (Huang et al., 2019; Hu et al., 2020). BM-MSCs inhibited liver fibrosis by decreasing the expression of IL-17A and IL-17RA and the serum levels of IL-17 in the liver (Farouk et al., 2018). Milosavljevic et al. also confirmed that MSC-conditioned medium (MSC-CM) promoted the expansion of CD4⁻FoxP3⁺-IL-10⁺-T regulatory cells and suppressed the proliferation of Th17 cells, which attenuated liver fibrosis (Dong et al., 2020).

Furthermore, BM-MSC transplantation promoted the activation of M2 macrophages expressing matrix metalloproteinase 13 (MMP13) and inhibited M1 macrophage activation. Meanwhile, MSCs reduced the expression of proinflammatory cytokines and increased the expression of anti-inflammatory cytokines (van der Helm et al., 2018; Luo et al., 2019; Yu et al., 2019). Increasing mitophagy and reducing mitochondrial ROS to restrict the inflammatory activation of macrophages may be critical mechanisms by which MSCs inhibit inflammation (Li et al., 2018). In response to oxidative stress, MSCs can transport depolarized mitochondria to macrophages through extracellular vesicles (Phinney et al., 2015). Mitochondrial transfer also promotes an anti-inflammatory macrophage phenotype by enhancing oxidative phosphorylation (Morrison et al., 2017). Existing studies also confirmed that hypoxia preconditioning and HIF-1 overexpression significantly improved MSC therapy (Martinez et al., 2017). MSCs cultured under hypoxic conditions presented an enhanced therapeutic effect on liver cirrhosis, which promoted macrophage polarity to an anti-inflammatory phenotype via prostaglandin E2 (PGE2) expression (Kojima et al., 2019). In summary, MSC treatment is emerging as a connecting bridge to drive immune and metabolic regulation and oxygen balance in the fibrotic microenvironment. We summarized the details of mechanism above in Figure 2B.

CONCLUSION AND FUTURE PERSPECTIVES

Mitochondrial dysfunction, hypoxia, inflammation, and metabolic reprogramming are widespread in fibrotic diseases. Here, we have reviewed the regulatory mechanism for immunometabolism and oxygen homeostasis in liver fibrosis (Figure 3) as well as potential novel targets for antifibrotic therapies. A special metabolic immune microenvironment mediated by oxygen is described, which deeply affects the balance of tissue damage and repair. The process of fibrosis is closely related to metabolic and immune disorders, which are usually induced by the destruction of oxygen homeostasis, including mitochondrial dysfunction, oxidative stress, and hypoxia signalling pathway activation. On the one
hand, destruction of oxygen homeostasis promotes oxidative stress and releases inflammatory mediators, forming a loop with an inflammatory response and cell damage. On the other hand, cell metabolic reprogramming affects the activation of immune cells and fibroblasts, epithelial mesenchymal transformation, and angiogenesis and further promotes the development of fibrosis. Furthermore, we noticed that hypoxia-induced metabolic reprogramming of immune cells and other fibrosis-related cells is an emerging research direction, but there is still a gap to be filled in the liver fibrosis field. In summary, as immunometabolism and oxygen homeostasis are relatively new research directions, the mechanism, function, and potential clinical application in liver fibrosis need and deserve further investigation.

REFERENCES

Agil, A., El-Hammadi, M., Jiménez-Aranda, A., Tassi, M., Abdo, W., Fernández-Vázquez, G., et al. (2015). Melatonin Reduces Hepatic Mitochondrial Dysfunction in Diabetic Obese Rats. *J. Pineal Res.* 59, 70–79. doi:10.1111/jpi.12241

Ai, W.-L., Dong, L.-y., Wang, J., Li, Z.-w., Wang, X., Gao, J., et al. (2018). Deficiency in Augmenter of Liver Regeneration Accelerates Liver Fibrosis by Promoting Migration of Hepatic Stellate Cell. *Biochim. Biophys. Acta (Bba) - Mol. Basis Dis.* 1864, 3780–3791. doi:10.1016/j.bbadis.2018.09.011

Ajaz, S., McPhail, M. J., Gnudi, L., Trovato, F. M., Mujib, S., Napoli, S., et al. (2021). Mitochondrial Dysfunction as a Mechanistic Biomarker in Patients with Non-alcoholic Fatty Liver Disease (NAFLD). *Mitochondrion* 57, 119–130. doi:10.1016/j.mito.2020.12.010

Alegre, F., Pelegrin, P., and Feldstein, A. (2017). Inflammomasomes in Liver Fibrosis. *Semin. Liver Dis.* 37, 119–127. doi:10.1055/s-0037-1601350

Ambade, A., Satishchandran, A., Saha, B., Gyongyosi, B., Lowe, P., Kody, K., et al. (2016). Hepatocellular Carcinoma Is Accelerated by NASH Involving M2 Macrophage Polarization Mediated by Hif-1α-induced IL-10. *Oncoimmunology* 5, e1221557. doi:10.1080/2162402X.2016.1221557

An, P., Wei, L.-L., Zhao, S., Sverdlov, D. Y., Jin, H., Lu, C., et al. (2016). Fate of Mesenchymal Stem Cells: Adipocytes or Osteoblasts? *Cell Death Differ* 23, 1128–1139. doi:10.1038/cdd.2015.168

Bouchez, C., and Devin, A. (2019). Mitochondrial Biogenesis and Mitochondrial Reactive Oxygen Species (ROS): A Complex Relationship Regulated by the cAMP/PKA Signaling Pathway. *Cells* 8, 287. doi:10.3390/cells8040287

Chen, Q., Chen, L., Wu, X., Zhang, F., Jin, H., Lu, C., et al. (2016). Dihydroartemisinin Prevents Liver Fibrosis in Bile Duct Ligated Rats by Inducing Hepatic Stellate Cell Apoptosis through Modulating the PI3K/Akt Pathway. *J. Atheroscler. Thromb.* 23, 220–231. doi:10.1002/jat.1478

Chen, Q., Shou, P., Zheng, C., Jiang, M., Cao, G., Yang, Q., et al. (2016). Fate Decision of Mesenchymal Stem Cells: Adipocytes or Osteoblasts? *Cell Death Dis.* 23, 1128–1139. doi:10.1038/cdd.2015.168

Chen, Y., Yousaf, M. N., and Mehal, W. Z. (2018). Role of Sterile Inflammation in Fatty Liver Diseases. *Liver Res.* 2, 21–29. doi:10.1080/2016.08.003

Cho, E.-H. (2018). Succinate as a Regulator of Hepatic Stellate Cells in Liver Fibrosis. *Front. Endocrinol.* 9, 455. doi:10.3389/fendo.2018.00455

Coppé, B. L., Kaska, S., and Wentling, C. (2012). Hypoxia-inducible Factor Activation in Myeloid Cells Contributes to the Development of Liver Fibrosis in Cholestatic Mice. *J. Pharmacol. Exp. Ther.* 341, 307–316. doi:10.1124/jpet.111.189340

Corcoran, S. E., and O'Neill, L. A. J. (2016). HIF-1α and Metabolic Reprogramming in Inflammation. *J. Clin. Invest.* 126, 3699–3707. doi:10.1172/jci84431

Cortes, E., Lachowski, D., Rice, A., Thorpe, S. D., Robinson, B., Yeldag, G., et al. (2019). Tamoxifen Mechanically Deactivates Hepatic Stellate Cells via the G Protein-Coupled Estrogen Receptor. *Oncogene* 38, 2910–2922. doi:10.1038/s41388-018-0631-3

Coto-Montes, A., Roga, J. A., Rosales-Corral, S., Fuentes-Broteo, L., Tan, D. X., and Reiter, R. J. (2012). Role of Melatonin in the Regulation of Autophagy and Mitophagy: a Review. *Mel. Cell Endocrinol.* 361, 12–23. doi:10.1016/j.mce.2012.04.009

Darby, I. A., and Hewitson, T. D. (2016). Hypoxia in Tissue Repair and Fibrosis. *Cell Tissue Res.* 365, 553–562. doi:10.1007/s00441-016-2461-3

Das, N., Mandala, A., Nazz, G., Sini, S., Jain, M., Bandypadhyay, D., et al. (2017). Melatonin Protects against Lipid-Induced Mitochondrial Dysfunction in Hepatocytes and Inhibits Stellate Cell Activation during Hepatic Fibrosis in Mice. *J. Pineal Res.* 62, e12404. doi:10.1111/jpi.12404

Dong, L., Pu, Y., Chen, X., Qi, X., Zhang, L., Xu, L., et al. (2020). hUCMSC-extracellular Vesicles Downregulated Hepatic Stellate Cell Activation and Reduced Liver Injury in S. Japonicum-Infected Mice. *Stem Cell Res Ther.* 11, 21. doi:10.1186/s13287-019-1539-8

El Agha, E., Kramann, R., Schreiber, R. K., Li, X., Seeger, W., Humphreys, B. D., et al. (2017). Mesenchymal Stem Cells in Fibrotic Disease. *Cell stem cell* 21, 166–177. doi:10.1016/j.stem.2017.07.011

Farouk, S., Sabet, S., Abu Zahra, F. A., and El-Ghor, A. A. (2018). Bone Marrow-Derived Mesenchymal Stem Cells Downregulate IL17A Dependent IL6/STAT3 Signaling Pathway in CCl4-Induced Rat Liver Fibrosis. *PloS one* 13, e0206130. doi:10.1371/journal.pone.0206130

Feng, J., Wang, C., Liu, T., Li, J., Wu, L., Yu, Q., et al. (2019). Procyandin B2 Inhibits the Activation of Hepatic Stellate Cells and Angiogenesis via the Hedgehog Pathway during Liver Fibrosis. *J. Cell Mol Med* 23, 6479–6493. doi:10.1111/jcmm.14543

Finkel, T. (2012). Signal Transduction by Mitochondrial Oxidants. *J. Biol. Chem.* 287, 4433–4440. doi:10.1074/jbc.R111.271999

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XL and QyZ: Writing the original draft. ZW: Collecting data. MZ: Conceptualization, Funding acquisition, Validation. QaZ: Conceptualization, Validation.

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Win, S., Than, T. A., Zhang, J., Oo, C., Min, R. W. M., and Kaplowitz, N. (2018). New Insights into the Role and Mechanism of C-Jun-N-Terminal Kinase Signaling in the Pathobiology of Liver Diseases. *Hepatology* 67, 2013–2024. doi:10.1002/hep.29689

Wong, B. W., Kuchnio, A., Bruning, U., and Carmeliet, P. (2013). Emerging Novel Functions of the Oxygen-Sensing Prolyl Hydroxylase Domain Enzymes. *Trends Biochemical Sciences* 38, 3–11. doi:10.1016/j.tibs.2012.10.004

Wu, L., Mo, W., Feng, J., Li, J., Yu, Q., Li, S., et al. (2020). Astaxanthin Attenuates Hepatic Damage and Mitochondrial Dysfunction in Non-alcoholic Fatty Liver Disease by Up-regulating the FGF21/PGC-1α Pathway. *Br. J. Pharmacol.* 177, 3760–3777. doi:10.1111/bph.15099

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., et al. (1999). Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator PGC-1. *Cell* 98, 115–124. doi:10.1016/s0092-8674(00)80611-x

Xu, H., Zhao, Q., Song, N., Yan, Z., Lin, R., Wu, S., et al. (2020). AdipoR1/AdipoR2 Dual Agonist Recovers Nonalcoholic Steatohepatitis and Related Fibrosis via Endoplasmic Reticulum-Mitochondria axis. *Nat. Commun.* 11, 5807. doi:10.1038/s41467-020-19668-y

Yang, X., Wang, Z., Kai, J., Wang, F., Jia, Y., Wang, S., et al. (2020). Curcumin Attenuates Liver Sinusoidal Endothelial Cell Angiogenesis via Regulating Glis-ProX1-HIF-1α in Liver Fibrosis. *Cell Profil.* 53, e12762. doi:10.1111/cpr.12762

Yoo, S. M., and Jung, Y. K. (2018). A Molecular Approach to Mitophagy and Mitochondrial Dynamics. *Mol. Cell* 41, 18–26. doi:10.1016/j.molcel.2018.2277

Yu, S., Cheng, Y., Zhang, L., Yin, Y., Xue, J., Li, B., et al. (2019). Treatment with Adipose Tissue-Derived Mesenchymal Stem Cells Exerts Anti-diabetic Effects, Improves Long-Term Complications, and Attenuates Inflammation in Type 2 Diabetic Rats. *Stem Cell Res Ther* 10, 333. doi:10.1186/s13287-019-1474-8

Yu, Y., Liu, Y., An, W., Song, J., Zhang, Y., and Zhao, X. (2018). STING-mediated Inflammation in Kupffer Cells Contributes to Progression of Nonalcoholic Steatohepatitis. *J. Clin. Invest.* 129, 546–555. doi:10.1172/jci121842

Yuen, V. W.-H., and Wong, C. C.-L. (2020). Hypoxia-inducible Factors and Innate Immunity in Liver Cancer. *J. Clin. Invest.* 130, 5052–5062. doi:10.1172/jci137553

Zhai, X., Qiao, H., Guan, W., Li, Z., Cheng, Y., Jia, X., et al. (2015). Curcumin Regulates Peroxisome Proliferator-Activated Receptor-γ Coactivator-1α Expression by AMPK Pathway in Hepatic Stellate Cells *In Vitro*. *Eur. J. Pharmacol.* 746, 56–62. doi:10.1016/j.ejphar.2014.10.055

Zhang, L., Zhang, Y., Chang, X., and Zhang, X. (2020). Imbalance in Mitochondrial Dynamics Induced by Low PGC-1α Expression Contributes to Hepatocyte EMT and Liver Fibrosis. *Cell Death Dis* 11, 226. doi:10.1038/s41419-020-2429-9

Zhang, Q., Rasoq, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., et al. (2010). Circulating Mitochondrial DAMPs Cause Inflammatory Responses to Injury. *Nature* 464, 104–107. doi:10.1038/nature08780

Zhang, Y., He, Y., Yu, H., Ma, F., Wu, J., and Zhang, X. (2015). Liquiritigenin Protects Rats from Carbon Tetrachloride Induced Hepatic Injury through PGC-1αPathway. *Evidence-Based Complement. Altern. Med.* 2015, 1–9. doi:10.1155/2015/69568

Zhao, P., Han, S.-N., Arumugam, S., Yousaf, M. N., Qin, Y., Jiang, J. X., et al. (2019). Digoxin Improves Steatohepatitis with Differential Involvement of Liver Cell Subsets in Mice through Inhibition of PKM2 Transactivation. *Am. J. Physiology-Gastrointestinal Liver Physiol.* 317, G387–g397. doi:10.1152/ajpgi.00054.2019

Zhuang, Q., Ma, R., Yin, Y., Lan, T., Yu, M., and Ming, Y. (2019). Mesenchymal Stem Cells in Renal Fibrosis: The Flame of Cytotherapy. *Stem Cell Int.* 2019, 1–18. doi:10.1155/2019/8387350

Zorov, D. B., Juhaszova, M., and Sollott, S. J. (2014). Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiol. Rev.* 94, 909–950. doi:10.1152/physrev.00626.2013

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GLOSSARY

ECM Extracellular matrix
HSCs hepatic stellate cells
DAMPs danger-associated molecular patterns
HIF-1α hypoxia inducible factor-1α
ATP adenosine-triphosphate
ROS reactive oxygen species
mtDNA mitochondrial DNA
NAFLD non-alcoholic fatty liver disease
PPAR γ peroxisome proliferator-activated receptor-gamma
PGC-1α peroxisome proliferator-activated receptor-gamma coactivator-1alpha
NRF nuclear respiratory factor
TFAM mitochondrial transcription factor A
CCI4 carbon tetrachloride
PSO pomegranate seed oil
EET-A epoxyeicosatrienoic acid-agonist
Mfn1 fusion proteins mitofusin 1
OPA1 optic atrophy 1
Drp1 dynamin-related protein 1
PM2.5 Particulate matter ≤2.5 μm
HKDC1 HK domain-containing 1
LA lipoic acid
mtDepo mitochondrial depolarization
BNIP3 Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3
mtDAMPs mitochondrial DAMPs
SOD superoxide dismutase
CAT catalase
GSH glutathione
NLRP3 NACHT, LRR and PYD domains-containing protein 3
MAPK mitogen-activated protein kinase
PHD prolyl hydroxylase domain
VHL von Hippel-Lindau
HRE hypoxia response element
ROCK1 Rho-associated coiled-coil-forming kinase 1
Cxc12 chemokine ligand 12
TGF-β transforming growth factor β
PROX1 prospero homebox protein 1
USP19 ubiquitin specific peptidase 19
LSECs liver sinusoidal endothelial cells
PPARγ peroxisomal proliferator receptor γ
GLUT1 glucose transporters 1
PKM2 pyruvate kinase isozymes M2
NPCs non-parenchymal cells
HFD high-fat diet
Apoe−/− apolipoprotein E-deficient
CLOCK circadian locomotor output cycles kaput
HCD high cholesterol diet
iNOS inducible nitric oxide synthase
NO nitric oxide
SI sterile inflammation
HMGB-1 high mobility group box-1
KC Kupffer cell
RAGE receptor for advanced glycation end-products
HCC hepatocellular carcinoma
PRRs pattern recognition receptors
APCs antigen-presenting cells
TLR9 Toll-like receptor 9
NFPs N-formyl peptides
NASH nonalcoholic steatohepatitis
IL interleukin
PA palmitic acid
APAP acetaminophen
miR-223 microRNA-223
cGAS cyclic GMP-AMP synthase
STING stimulator of interferon genes
NF-κB nuclear factor-κB
IFN-I type I interferon
FPRs formyl peptides receptors
SIRS systemic inflammatory response syndrome
IRI ischemia/reperfusion injury
PDGF-B platelet-derived growth factor-B
MCD methionine-choline-deficient
MCP-1 monocyte chemoattractant protein-1
APOBEC3B apolipoprotein B mRNA editing enzyme catalytic subunit 3B
DNases deoxyribonucleases
HRGP histidine-rich glycoprotein
ALR augmenter of liver regeneration
DHA dihydroartemisinin
GPER G protein-coupled estrogen receptor
p-ERK phosphorylation of extracellular signal-regulated kinase
VEGF vascular endothelial growth factor
MSCs mesenchymal stem cells
MSC-CM MSC-conditioned medium
MMP13 matrix metalloproteinase 13
PGE2 prostaglandin E2