Emerging Role of High-Mobility Group Box 1 (HMGB1) in Liver Diseases

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Damage-associated molecular pattern (DAMP) molecules are essential for the initiation of innate inflammatory responses to infection and injury. The prototypic DAMP molecule, high-mobility group box 1 (HMGB1), is an abundant architectural chromosomal protein that has location-specific biological functions: within the nucleus as a DNA chaperone, within the cytosol to sustain autophagy and outside the cell as a DAMP molecule. Recent research indicates that aberrant activation of HMGB1 signaling can promote the onset of inflammatory and autoimmune diseases, raising interest in the development of therapeutic strategies to control their function. The importance of HMGB1 activation in various forms of liver disease in relation to liver damage, steatosis, inflammation, fibrosis, tumorigenesis and regeneration is discussed in this review.

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INTRODUCTION

The high-mobility group (HMG) chromosomal proteins were discovered in mammalian cells in 1973 and named according to their high electrophoretic mobility in polyacrylamide gels (1). High-mobility group box 1 (HMGB1), as a member of the HMG family, has location-specific biological functions: within the nucleus as a DNA chaperone, within the cytosol to sustain autophagy and outside the cell as a DAMP molecule. HMGB1 is essential for life because the absence of this protein is postnatally lethal; newborn knockout mice succumb to hypoglycemia (2). Although initial studies demonstrated HMGB1 as a late mediator of lethal systemic inflammation, recent findings indicate that HMGB1 has an important role in noninfectious inflammation, such as autoimmunity, cancer, trauma and ischemia/reperfusion (I/R) injury (3–5). During the past decade, studies in both patients and animal models have established that HMGB1 represents a potential biomarker and novel therapeutic target in certain diseases. There have been important advances recently in the understanding of the structure and function of HMGB1 (Figure 1). In this review, we will summarize the basics of HMGB1 and focus on the current understanding of connections between HMGB1 and liver disease and its potential as a therapeutic target.

HMGB1 STRUCTURE

HMGB1 is encoded on human chromosome 13q12-13 and consists of 215 amino acids. HMGB1 is a highly conserved protein containing two DNA-binding domains (HMGB A and B) and a natively charged C tail (for transcription stimulation) domain (Figure 1A). Both the HMGB1 A and B boxes are about 75–80 amino acids long and are formed by two short and one long α-helix that, upon folding, produce an L- or V-shaped three-dimensional domain structure (6–8). The B box has been identified as a function domain, which can be recognized by toll-like receptor (TLR)-4 and trigger the release of proinflammatory cytokines (9). In comparison, the purified recombinant A box has antiinflammatory properties in vivo and in vitro (10). Amino acids 150–183 of HMGB1 are responsible for the receptor for advanced glycation end products (RAGE) binding (11), whereas amino acids 89–108 of HMGB1 are responsible for binding to TLR4 (9). Structurally, cytokine-HMGB1 (the form with cytokine activity) has a disulfide bridge between cysteine residues 23 and 45 and a reduced cysteine residue 106, whereas chemotaxis-HMGB1 (the chemoattractant form) is completely reduced (12,13).
HMGB1 FUNCTION

In 1978, HMGB1 was identified as a nonhistone chromosomal protein involved in DNA binding and bending and participating in regulation of DNA structure (14). In the last decades, accumulated evidence has demonstrated that HMGB1 plays a critical role in controlling multiple DNA signaling pathways (for example, gene transcription, genetic recombination, and DNA damage repair) by protein–DNA or protein–protein interactions. HMGB1 interacts with and enhances the activities of a number of transcription factors, including p53, p73, the retinoblastoma protein (RB), members of the Rel/NF-κB family, and estrogen receptor (ER). Once released, HMGB1 binds to various receptors to activate DAMP signaling involved in multiple cellular processes. Cytoplasmic HMGB1 protein binds with beclin 1 to induce autophagy. Membrane HMGB1 promotes neurite outgrowth and platelet activation.

HMGB1 RELEASE

Extracellular HMGB1 is derived either by active secretion by innate immune cells (for example, macrophages, monocytes, and dendritic cells [DCs]) or by passive release by dead, dying, or injured cells (for example, cancer cells and hepatocytes) (Figure 2). Depending on the inducing stimulus, the mechanism of HMGB1 secretion and release can vary. In response to exogenous pathogen-associated molecular patterns (PAMPs) such as endotoxin or cytosine-phosphate-guanine [CpG]-DNA, or endogenous inflammatory stimuli (for example, tumor necrosis factor [TNF], interferon [IFN]-γ, hydrogen peroxide, or DAMP molecules), HMGB1 is modified by different posttranscriptional modifications (for example, acetylation or phosphorylation) and actively released by innate immune cells (22,23). HMGB1 lacks a leader signal sequence and cannot be released via the classic endoplasmic reticulum–Golgi-dependent secretory pathway. Instead, HMGB1 can be secreted through noncanonical, secretory, lysosome-mediated exocytosis in active immune and inflammatory cells (24). In addition, inflammasome, a large caspase-1–activating multiprotein complex, was recently shown to regulate HMGB1 release in immune and inflammatory cells (25). Notably, double-strand RNA (dsRNA)-dependent protein kinase–mediated inflammasome activation is an essential regulator of HMGB1 secretion (26). HMGB1 release has been
considered a feature of necrosis but not apoptosis (27). Recent studies suggest that HMGB1 can also be released by apoptotic cells at a late stage (a stage that can be called secondary necrosis) and induce immune tolerance (28). Autophagy can regulate both active secretion and passive release of HMGB1 in macrophages and cancer cells, respectively (20,29). The differences observed in HMGB1 release during death may reflect the experimental systems used, including the cell lines and death inducers.

**HMGB1 RECEPTORS**

The receptors used by HMGB1 are not entirely clear and may vary by context. These receptors include RAGE, the TLRs, Mac-1, syndecan-1 (CD138), phosphatase-tyrosine phosphatase (PPTP)-ζ/β, CD24, chemokine (C-X-C motif) receptor 4 (CXCR4), T-cell immunoglobulin mucin-3 (TIM-3) and possibly others (Figure 1C). RAGE, a member of the immunoglobulin superfamily of cell surface molecules expressed on a variety of cell types, has a high affinity for HMGB1. HMGB1 interaction with RAGE can mediate cell proliferation, growth and migration by activating intracellular signals such as nuclear factor (NF)-κB and the mitogen-activated protein kinase (MAPK) pathway (30,31). In addition to RAGE, the importance of TLRs has been demonstrated in HMGB1 signaling pathways. TLRs are highly conserved proteins and important pathogen-recognized patterns both in innate and adaptive immunity. Three of the TLRs have been reported to be involved in HMGB1 signaling: TLR2, TLR4 and TLR9. Signaling by activation of these TLRs culminates in NF-κB and MAPKs that regulate gene expression of various immune and inflammatory mediators (32). Recently, it was suggested that bacterial DNA or other contaminants in bacterially expressed HMGB1 may activate both TLR9 and RAGE (33). In addition, CD24 and TIM-3 act as negative receptors and inhibit immune activity of HMGB1 in macrophages and tumor-associated DCs, respectively (Figure 2) (34,35). HMGB1 also promotes recruitment of inflammatory cells to damaged tissue by forming a complex with the chemokine CXCL12 and signaling via CXCR4 independent of RAGE and TLR4 (36). Taken together, these studies support the possibility that tissue and cell-type specific roles for HMGB1 receptors exist. Interplay between HMGB1 receptors within the same tissue and cells remains largely unknown.

**HMGB1 IN LIVER DISEASES**

The liver plays a key role in integration of immune responses after severe systemic insult such as trauma or surgery. In the liver, TLRs and RAGE are expressed by parenchymal and nonparenchymal cell types, which is likely biologically important, since these receptors can initiate innate immune cascades through the recognition of HMGB1 in the pathogenesis of liver disease as discussed below.

**HMGB1 AND LIVER I/R**

I/R injury refers to a set of deleterious phenomena that emanate from the tem-
Temporary deprivation of circulation and subsequent restoration of oxygen supply. There are two different forms of hepatic I/R: warm I/R and cold I/R, which differ with respect to the hepatic blood flow stage. Warm I/R can occur in low-flow states, surgery, injury and trauma. In contrast, cold I/R occurs in the time during organ preservation before transplantation. Local cellular damage causes the release of DAMP molecules and subsequent systemic sterile inflammatory response, two characteristics in the pathophysiology of hepatic I/R injury (37). The involvement of HMGB1 in warm I/R injury was first described in 2005 (38,39). HMGB1 was rapidly released into circulation by hepatocytes in I/R. TLR4 plays a central role in liver I/R (Figure 3). TLR4 is required for HMGB1 release and protects hepatic I/R damage in TLR4 wild-type mice, but not TLR4-deficient mice (38). In addition, TLR4 from parenchymal cells but not myeloid cells and DCs also regulates hepatic I/R-induced HMGB1 release by activation of reactive oxygen species and calcium/calmodulin-dependent protein kinase (CaMK) signaling pathway (38,40). Treatment with CaMK inhibitor (KN93) and antioxidant N-acetylcysteine inhibit HMGB1 release and protect against liver I/R injury in animal models. CD14 is known to participate in ligand recognition by the TLR4 receptor complex and is involved in both the injury and the inflammation induced by liver I/R (41). However, the release of HMGB1 in liver I/R did not require CD14, whereas the recognition of HMGB1 needs CD14 (41). TLR9 (42) and RAGE (43) inhibition also confer protection from liver I/R injury, suggesting that HMGB1 may interact with multiple receptors to mediate warm liver I/R injury.

Figure 3. HMGB1-TLR4 signaling mediates liver I/R injury. HMGB1 is an early mediator of injury and inflammation in liver I/R, and TLR4 is the major receptor that is involved in the process by which the JNK MAPK/NF-κB pathway is activated. The release of HMGB1 in liver I/R requires TLR4 but not CD14. Different strategies of HMGB1-TLR4 pathway inhibition as indicated have been shown to reduce liver I/R injury.

In addition, a range of mechanisms such as inflammasome activation (44), decreased histone deacetylase activity (45) and upregulation of nuclear interferon regulatory factor 1 (46) have been identified to contribute to HMGB1 release in warm liver I/R damage. Recent studies show that Bβ15-42 (the fibrin-derived peptide), PNU-282987 (a selective α7 nicotinic acetylcholine receptor agonist), EPC-K1 (the vitamin E derivative), melatonin, cisplatin and glycyrrhizin attenuate warm liver I/R damage partly through inhibition of HMGB1 release (47–52). Interestingly, pretreatment of mice with HMGB1 protein significantly decreased liver I/R injury through upregulation of IL-1R-associated kinase-M, a negative regulator of TLR4 signaling (53). Thus, extracellular HMGB1 may have a dual role in regulation of hepatic I/R injury, which depends on its posttranslational modifications and receptors.

HMGB1 AND LIVER TRANSPLANTATION

Liver transplantation remains the primary treatment for patients with end-stage liver disease, including cirrhosis. During liver transplantation, the stage of cold preservation after organ harvest often results in predominantly sinusoidal cell injury and a warm phase of organ reperfusion, in which the expected ischemic injury with disruption of multiple cellular metabolic processes is initiated (54,55). A second type of injury occurs on reperfusion and consists of a rapid inflammatory response (56). The extent of damage is associated with the length of ischemia time and mechanical stress. TLR4 has been implicated in cold I/R injury during liver transplantation. A recent clinical study indicates that HMGB1 is a useful biomarker of hepatocellular injury in human liver transplantation (57). They found the following: (a) reperfusion after cold ischemia in human liver transplantation was related to extensive HMGB1 efflux from the graft (57); and (b) the level of HMGB1 expression and release in hepatocytes correlated with peak postoperative alanine aminotransferase (ALT) levels (57). In addition to
cold I/R injury, immunologic tolerance and rejection are major impediments to successful liver transplantation. However, whether HMGB1 is linked to the subsequent immunologic tolerance and graft rejection and its precise role and mechanism remain unknown. Redox state and cell death form have been reported to determine whether HMGB1 is tolerogenic or immunogenic in macrophages and DCs (28).

HMGB1 AND VIRAL HEPATITIS

At least five different hepatitis viruses (hepatitis A to E viruses) cause liver disease in humans. All five cause acute hepatitis, whereas hepatitis B (HBV), C (HCV), and D (HDV) viruses can also lead to persistent infection and chronic hepatitis. HBV is the major cause of chronic hepatitis, cirrhosis and liver cancer worldwide. Most HBV infections are silent; thus, a significant number of persistently infected individuals remain unaware of infection, even for decades, which has become a major public health problem (58). Chronic infection with either HBV or HCV can result in inflammation and oxidative stress. A prolonged fibrotic response resulting in cirrhosis is also common in both infections, which is accompanied by the appearance of localized hypoxia, rearrangement of tissue architecture (epithelial–mesenchymal transition) and angiogenesis (59). Thus, viral hepatitis confers a risk of developing a chronic infection that can lead to liver fibrosis and eventually evolve into liver cirrhosis and hepatocellular carcinoma (60). Although multiple signaling networks are responsible for coordinating the inflammatory and immune response during virus hepatitis, HMGB1 is critical in initiating and mediating these effects. HMGB1 is translocated from the nucleus to the cytoplasm and subsequently is released into the extracellular milieu by HCV or HBV infection (61–63). Extracellular HMGB1 causes chronic inflammation by activation of NF-κB pathways or results in TLR4-dependent interferon antiviral response. Serum HMGB1 level is increased in several chronic liver diseases. HMGB1 induces HSC activation by TLR4-MyD88/MAPK-NF-κB pathways. HMGB1 promotes the expression of collagen and α-SMA in HSCs and results in accumulation of excess extracellular matrix and liver fibrosis. Extracellular HMGB1 binds to its receptors (e.g., TLR4, TLR9 and RAGE) to induce inflammasome activation and proinflammatory cytokine release, which is essential for tumor growth, invasion and metastasis.

HMGB1 AND NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD), one of the most common causes of chronic liver disease in developed countries, is caused by an accumulation of lipid deposits in the liver. NAFLD includes a wide variety of liver damage, ranging from steatosis (only fat accumulation, also called fatty liver), to nonalcoholic steatohepatitis (fatty infiltration associated with inflammation and
liver injury), to advanced fibrosis and cirrhosis (permanent damage/injury to the liver). The initial theory for the pathogenesis of NAFLD was the “two-hit” hypothesis (66). The “first hit,” hepatic triglyceride accumulation (also called steatosis), sensitizes the liver to injury by “second hits,” such as those from cytokines, endotoxin, adipokines, mitochondrial dysfunction and oxidative stress, which result in de novo lipogenesis and increased release of free fatty acid and abnormal lipid deposition in the liver (67). Emerging evidence suggests that HMGB1-TLR4-MyD88 signaling is involved in the progression of NAFLD in the early stage (Figure 4B) (68). Knockout of TLR4 and its cytosolic adapter protein MyD88 in mice decreases serum HMGB1 level and suppresses high-fat diet–induced liver dysfunction. The accumulation of free HMGB1 in the plasma may further enhance inflammation and liver damage. Treatment with HMGB1-neutralizing antibody inhibits free fatty acid–induced proinflammatory cytokines (for example, TNF and IL-6) production (68). These studies indicate that HMGB1 serves as an important mediator to accelerate local liver damage and systemic inflammation during the early stage of NAFLD.

HMGB1 AND LIVER FIBROSIS

Liver fibrosis is the final common pathway for a number of chronic liver diseases. Regardless of the underlying etiology, hepatic fibrosis is a kind of liver injury and scar repair, characterized by the accumulation of excess extracellular matrix (ECM), a reduction of ECM-removing matrix metalloproteinase (MMP) and an upregulation of tissue inhibitors of MMP (69). Hepatic stellate cells (HSCs) are quiescent cells in the perisinusoidal space in the liver that facilitate hepatocyte interactions in the process of liver fibrosis via the release of soluble inflammatory factors and the production of ECM (69). HMGB1 is involved in the development and progression of liver fibrosis (Figure 4B) (60). Recombinant HMGB1 protein markedly stimulates HSC growth, promotes α-smooth muscle actin (α-SMA) expression and inhibits MMP-2 activity (70). In a rat model of hepatic fibrosis, the level of HMGB1 is upregulated, and its expression is closely correlated with the deposition of collagen. Suppression of HMGB1 expression by small interfering RNA (siRNA) significantly inhibits synthesis of α-SMA and collagen (types I and III) in HSCs (71). In addition, HMGB1 released during the rejection phase of orthotopic liver transplantation has the ability to activate HSCs and exhibit profibrogenic effects on liver grafts (70). HMGB1 also has a synergistic effect with transforming growth factor β1 (TGF-β1) to stimulate expression of fibrogenic protein (for example, collagen α2 and α-SMA) in HSCs (72). HMGB1 promotes the proliferation/migration of HSCs and liver fibrosis partly via TLR4-MyD88 and the MAPK–NF-κB pathway (Figure 4B) (72,73). These studies have demonstrated that HMGB1 and its profibrotic function may be effective targets to treat liver fibrosis.

HMGB1 AND HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is one of the most common forms of cancer in the world, especially in Asia and Africa (58). Approximately 90% of HCCs are preceded by chronic liver disease, hepatic fibrosis and cirrhosis (Figure 4C). HCC is a typical inflammation-related carcinoma, commonly arising in a damaged organ, featuring extensive inflammation and fibrosis. Different players, including immune cells, hepatic stellate cells and macrophages, react to liver injury by producing cytokines and components of the ECM, which promote angiogenesis and survival of damaged hepatocytes or cancer stem cells. HMGB1 is dysfunctional in various tumors and plays a context-dependent role in the regulation of tumor development and therapy (74). Extracellular HMGB1-induced chronic inflammation within the tumor microenvironment favors tumor survival, proliferation, metastasis and angiogenesis. Expression of HMGB1 is closely correlated with pathological grade and distant metastases of liver cancer, and knockdown of HMGB1 inhibits liver cancer growth and metastasis (75,76). Serum HMGB1 levels were significantly higher in HCC and HBV patients and were associated with clinicopathological features and outcome in HCC patients (64,77). Ethyl pyruvate, a potent inhibitor of HMGB1 release, can suppress tumor growth in liver metastasis models of colon cancer (78). HMGB1 and its receptor RAGE were shown to modulate the proliferation and cell cycle of human HCC cell lines in vitro (79).

Hypoxia-induced HMGB1 release in HCC cell lines can activate TLR4- and RAGE-signaling pathways to induce activation of the inflammasome and secretion of IL-1β and IL-18, which, in turn, promote HCC invasion and metastasis (Figure 4C) (80). A recent study indicated that activation of PAMP-TLR4 signaling contributes to injury/inflammation-driven tumor promotion and progression in late stages of hepatocarcinogenesis (81). Thus, TLR4 activation by PAMPs and DAMP molecules has a critical role in HCC pathogenesis.

HMGB1 AND DRUG-INDUCED LIVER INJURY

Drug-induced liver injury, also known as hepatotoxicity, refers to liver injury caused by drugs or other chemical agents and represents a special type of adverse drug reaction. Acetaminophen (APAP) overdoses are currently the most frequent cause of acute liver failure in the United States (82). Hepatocyte necrosis, apoptosis and innate immune activation have been defined as the dominant features of the toxicological response associated with APAP. HMGB1 has been reported as a circulating mechanistic indicator of cell death in animal and clinical studies on APAP-induced hepatotoxicity (83–85). HMGB1 also becomes a sensitive serum biomarker of APAP-induced acute liver injury (83–85). APAP-induced HMGB1 release contributes to innate immune activation during liver injury (27,86). For example, the HMGB1–TLR4–IL-23–IL-
17A signaling pathway mediates interplay between macrophages and γδ T cells, which contributes to APAP-induced liver inflammation (87). The change of cysteine redox isoforms of HMGB1 is an important mechanism to regulate HMGB1 activity during inflammation, immunity and cell death (88). Caspase-dependent oxidation of HMGB1 prevents inflammatory response in mice treated with APAP (85). It is also important to note that the release of the specific HMGB1 isoform such as acetylated HMGB1 is related to drug-induced liver injury prognosis in the patient and mouse model (89,90). Blocking HMGB1 release or activity by ethyl pyruvate and HMGB1-neutralizing antibody prevents APAP-induced hepatotoxicity and restores liver structure by inhibition of oxidative injury and inflammation (91,92). In addition, HMGB1 cytoplasmic translocation and release during tissue damage and cell death promotes pathological processes in several drug-induced acute liver failures (93,94).

**HMGB1 AND LIVER REGENERATION**

Compared with other organs, liver has the greatest capacity to regenerate in response to a variety of stimuli. This regeneration capacity is critical for survival after partial hepatectomy and acute and chronic liver damage. There are many different factors such as growth factors, cytokines, DNA synthesis and cell cycle progression that affect liver regeneration (95). Growing evidence indicates that the expression and release of HMGB1 is associated with liver regeneration after various liver injuries (63,92). Blocking HMGB1 activity by HMGB1-neutralizing antibody increases cyclin D1 expression and improves hepatocyte regeneration in APAP-injected mice (92). Inhibition of HMGB1 release by glycyrrhizin also improves hepatocyte regeneration after I/R in rats (48). These studies suggest that extracellular HMGB1 limits liver regeneration, although the potential mechanism of this association still remains largely unknown.

**HMGB1-TARGETING THERAPEUTIC STRATEGIES**

As discussed below, several strategies have been shown to protect against HMGB1 release and biological function in preclinical animal studies (Table 1) (96); however, these approaches have not yet been performed in clinical studies.

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HMGB1 Neutralizing Antibody

Currently used neutralizing antibodies include the monoclonal mouse anti-HMGB1 DPH1.1 antibody (HMGBiotech, Milan, Italy), the monoclonal mouse anti-HMGB1 2G7 antibody (Kevin Tracey’s lab, The Feinstein Institute for Medical Research, Manhasset, NY, USA) and the anti-HMGB1 chicken IgY polyclonal antibody (IBL International, Gunma, Japan).

A Box Protein

A recombinant fusion protein of the A box with thrombomodulin or a truncated HMGB-1–derived A box protein can effectively inhibit HMGB1 cytokine activity.

RNA interference (RNAi)

Suppression of HMGB1 expression through shRNA or siRNA can directly reduce intracellular and extracellular levels of HMGB1. However, intracellular and extracellular HMGB1 may have different roles in cancer and inflammation.

Adsorption

Hemoperfusion therapy using a cellulofine sulfate bead column that adsorbs HMGB1 reduces serum HMGB1 to decrease reperfusion injury of the rat liver.

Chemicals

Several chemicals and bioactive substances affect HMGB1 expression and release, including molecular hydrogen, quercetin, statins (atorvastatin or atorvastatin A II A), dexamethasone, gold sodium thiomolate, chloroquine, ethyl pyruvate, glycyrrhizin and stearoyl lysophosphatidylcholine. Additionally, certain Chinese medicinal herbs, such as Danshen (Angelica sinensis), Danshen (Salvia miltiorrhiza), green tea (Camellia sinensis) and Mung bean (Vigna radiata) can inhibit HMGB1 release and activity.

HMGB1 Receptor and Signaling Pathway Inhibition

The inhibition of HMGB1-related receptors (for example, RAGE and TLRs) and downstream signaling molecules can inhibit HMGB1 activity. For example, pathological effects mediated via RAGE are physiologically inhibited by soluble RAGE (sRAGE).

CONCLUSIONS AND PERSPECTIVES

As a basic pathological process of various diseases, inflammation contributes to progressive liver disease ranging from liver damage to fibrosis, as well as tumorigenesis (97,98). HMGB1 released by damaged liver tissue can lead to prolonged inflammatory and immune responses and influence the progression of liver disease. Despite significant progress, the mechanisms underlying HMGB1 release, the surface receptors that interact with HMGB1 and the intracellular signal transduction pathways of HMGB1 remain relatively poorly defined. Large human patient populations with well-defined clinical disease characteristics and more animal liver disease models are needed to more thoroughly characterize HMGB1 function in liver disease. In addition, although HMGB1-specific antagonists have been proven effective in preclinical animal models of diverse conditions mentioned above, this approach has not yet been performed in patients in well-controlled clinical studies.

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DISCLOSURE

The authors declare that they have no competing interests as defined by Molecular Medicine, or other interests that might be perceived to influence the results and discussion reported in this paper.

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HMGB1 AND LIVER DISEASES