Research Progress on the Role and Mechanism of IL-37 in Liver Diseases

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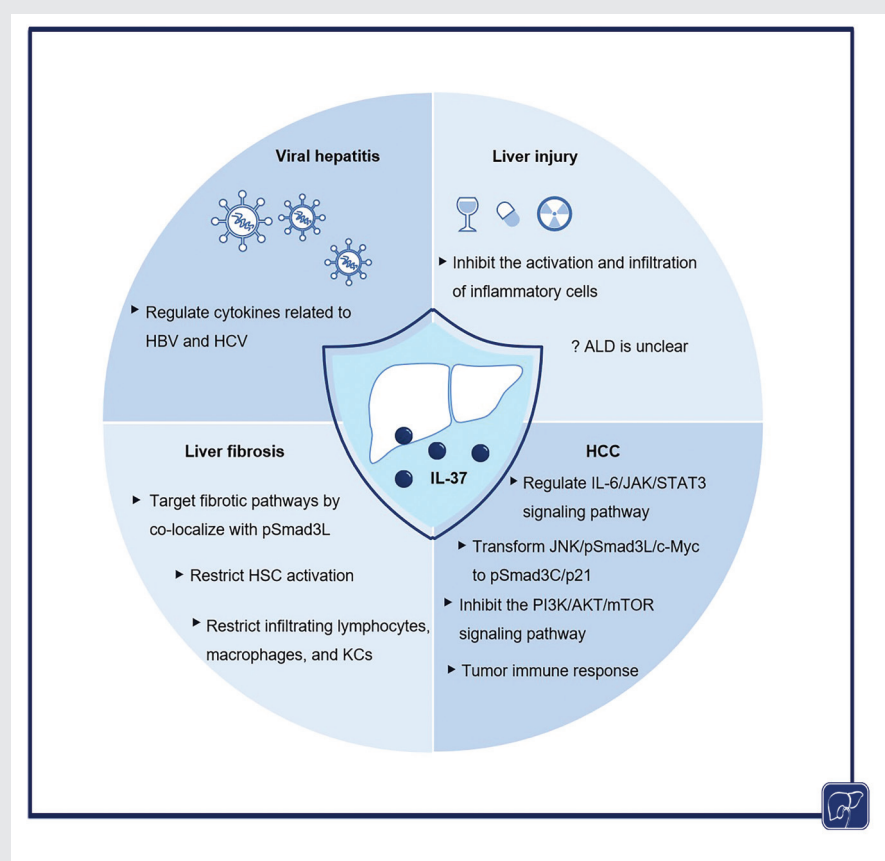
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Graphical Abstract

Abstract

Cytokines are important components of the immune system that can predict or influence the development of liver diseases. IL-37, a new member of the IL-1 cytokine family, exerts potent anti-inflammatory and immunosuppressive effects inside and outside cells. IL-37 expression differs before and after liver lesions, suggesting that it is associated with liver disease; however, its mechanism of action remains unclear. This article mainly reviews the biological characteristics of IL-37, which inhibits hepatitis, liver injury, and liver fibrosis by inhibiting inflammation, and inhibits the development of hepatocellular carcinoma (HCC) by regulating the immune microenvironment. Based on additional evidence, combining IL-37 with liver disease markers for diagnosis and treatment can achieve more significant effects, suggesting that IL-37 can be developed into a powerful tool for the clinical adjuvant treatment of liver diseases, especially HCC.

Keywords

► IL-37
► hepatitis
► liver injury
► liver fibrosis
► HCC

Liver disease has become a serious global public health problem owing to its high prevalence and poor long-term clinical treatment outcomes. With lifestyle changes, the number of patients with alcoholic and nonalcoholic fatty liver diseases (NAFLDs) caused by factors, such as alcoholism and obesity, has markedly increased.[1] However, owing to a lack of awareness about the dangers of liver disease and low treatment rates, most patients are diagnosed with cirrhosis or the late stages of liver cancer. As a result, approximately 2 million deaths occur every year due to liver disease. Moreover, the incidence of cirrhosis and hepatocellular carcinoma (HCC) is increasing.[2] At present, HCC results from the long-term accumulation of gene mutations.[3] Cancer cells differ from normal tissues and cells before tumor formation. Under physiological conditions, the human immune system recognizes these differences and activates an immune response to eradicate the cancer cells.[4] Unfortunately, tumor cells use various means to deceive immune cells and escape immune surveillance, including M2 macrophages, myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and Th2 cytokines to suppress tumor surveillance.[5] Therefore, restoring immune system homeostasis and enhancing liver immune function are potential therapeutic strategies for the treatment of liver diseases.

Cytokines are small, secreted proteins produced when immune cells, including myeloid cells and lymphocytes, are activated. Cytokines can modulate immune and inflammatory responses, thereby playing a key role in the development of the immune system. Cytokines are involved in the regulation of various liver diseases, such as hepatitis, liver injury, liver fibrosis, and HCC, and provide effective information for the diagnosis and treatment of liver diseases.[6,7] At the Second International Lymphokine Conference in Interlaken, Switzerland, interleukins were named.[8] IL-37, an anti-inflammatory cytokine in the IL-1 family, was discovered in 2000 in a computer sequencing study.[9] IL-37 was later named IL-1F7b, the seventh member of the IL-1 cytokine family, and recently renamed IL-37.[10,11] According to some researchers, IL-37 may be involved in the immune response, inflammatory diseases, or cancer when studying discrete cell populations, including plasma and tumor cells.[10] Dinarello and coworkers transfected murine macrophages with IL-37 and found that the proinflammatory cytokines induced by toll-like receptors (TLRs) were inhibited. This study was the first to identify IL-37 as a natural inhibitor of congenital inflammation and the innate immune response.[11]

In preclinical studies, many cytokines demonstrated the potential to treat liver disease, some of them are currently in clinical trials. These cytokines include recombinant IL-22 protein for the treatment of patients with severe alcoholic hepatitis and IL-1 inhibitors.[6] IL-6 exerts an obvious liver-protective function; however, due to the widespread expression of its receptor, it has many potential side effects in the treatment of liver diseases, leading to its poor clinical application.[12] The expression of IL-37 is low in healthy human tissues and cells[11]; however, when the body is stressed, its expression level increases to play an anti-inflammatory role. Although IL-37 has not been tested in clinical trials, basic experiments have shown that it plays a protective role in liver diseases and can be a powerful tool for the clinical adjuvant treatment of liver diseases.

Biological Characteristics of IL-37

The human IL-37 gene is located on chromosome 2, with a length of 3.617 kb and a molecular weight of approximately 17 to 25 kDa. The structure of this gene is similar to that of the IL-1 family (IL-1F), which consists of 12β tubular lines.[13] To date, five gene subtypes of IL-37a to IL-37e have been identified, which are encoded by six exons; however, the specific activity and relative abundance of each subtype are not clear. Among these, exons 4, 5, and 6 encode the sequence required for β-trefoil secondary structure, which is a characteristic of the IL-1 family. IL-37a, IL-37b, and IL-37d share exons 4, 5, and 6, indicating that IL-37a, IL-37b, and IL-37d may encode functional proteins. However, IL-37c and IL-37e cannot encode β-trefoil secondary structures due to the lack of exon 4, but may encode nonfunctional proteins.[14]

Each subtype of IL-37 is converted from inactive precursor peptides that regulate each other to form a relatively stable

state. Caspase-1 is the major cleavage enzyme responsible for the maturation of the IL-37 precursor and extracellular export of active cytokines.[15] The cleavage site for caspase-1 is currently thought to be located in the coding sequence of exon 1 between residues D20 and E21; however, an alternative cleavage site exists in exon 2 between amino acid residues F45 and V46.[14,16] Among them, IL-37a encodes a unique N-terminus, because it contains exon 3 with the lack of exons 1 and 2. IL-37b is the most complete one and has the largest molecular weight of the five isoforms, which include five of the six exons of the IL-37 gene (except exon 3). Therefore, IL-37b is the most active, abundant, and studied.[17] Although IL-37c has no biological function, it shares an N-terminal sequence (exons 1 and 2) with IL-37b and IL-37d and can compete with IL-37b and IL-37d precursors for the same cleavage enzyme targets. Accordingly, production of the precursor IL-37c downregulates IL-37b and IL-37d.[14] IL-37e, which also has no biological function, lacks exon 2 and cannot compete with IL-37b, despite sharing the cleavage site of caspase-1 with IL-37b at exon 1. In addition, IL-37e can only partially downregulate the production of IL-37b.[14]

In human tissues, IL-37 is mainly expressed in the liver, bone marrow, lungs, placenta, thymus, uterus, lymph nodes, testis, and tumor tissues.[18] On the cellular side, IL-37 is mainly expressed in tonsillar B cells, circulating monocytes, dendritic cells (DCs), tissue macrophages, and plasma cells. In addition, IL-37 mRNA contains unstable sequences in its coding region, and the transcription level of IL-37 mRNA in normal human monocytes is very low. The expression level of IL-37 in immune cells, such as monocytes, DCs, and T cells, increases rapidly after proinflammatory stimuli; however, only myeloid DCs secrete IL-37 under homeostasis.[19,20] Interestingly, IL-37 is the only member of the IL-1 family whose mouse homolog has not been identified, possibly due to the evolutionary loss of the gene.[11] Notably, the expression levels of the different shear isomers of IL-37 in human tissues also vary. IL-37a to IL-37c is expressed in the central lymphoid organ, liver, lung, testis, placenta, uterus, colon, and immune cells (natural killer [NK] cells, monocytes, stimulatory B cells). IL-37a is the only isoform expressed in the brain, IL-37b is expressed in the kidney, IL-37c is a specific isoform of the heart, and IL-37d and IL-37e are detected only in the bone marrow and testis.[14]

Anti-inflammatory Properties of IL-37

Inflammation and immunity are regulated by cytokines, which can serve as effective therapeutic targets for liver diseases.[21,22] In the IL-1 family comprising IL-37, in addition to IL-37, the cytokines with anti-inflammatory effects include IL-1Ra, IL-36Ra, and IL-38. However, IL-1Ra, IL-36Ra, and IL-38 are receptor antagonists. IL-37 has a wide range of protective effects and is the only anti-inflammatory cytokine belonging to the IL-1 family. The expression level of IL-37 in the peripheral blood of healthy individuals is very low; however, its level increases in many disease models (such as rheumatoid arthritis, oral squamous cell carcinoma, systemic lupus erythematosus, and hepatocellular). Transgenic

mouse IL-37 is mainly expressed in hepatocytes rather than that in immune cells,[23] which is beneficial for controlling inflammation and protecting the liver.

As the only cytokine in the IL-1 family with anti-inflammatory effects, IL-37 can exert dual anti-inflammatory activities inside and outside the cells. IL-37 is encoded by a gene located on chromosome 2q12–13, which is very close to the regulatory regions of the IL-1α and IL-1β genes. This specific location is closely related to the role of IL-37 in the inhibition of inflammatory responses.[24] In cells, inflammation stimulates the production of the IL-37 precursor and activates caspase-1. Caspase-1 cleaves the IL-37 precursor, most of which forms mature IL-37, and secretes it outside the cell. A small portion combines with phosphorylated Smad3 to form complexes that translocate to the nucleus and inhibit the expression of inflammatory genes. Cellular proteases cleave the precursor IL-37 extracellularly. IL-37 binds to IL-18 receptor α (IL-18Rα) to recruit the anti-inflammatory orphan member of the IL-1 receptor family, IL-1 receptor 8 (IL-1R8), to form a complex. IL-1R8, also called SIGIRR (single immunoglobulin interleukin-1 receptor-related protein) or toll/IL-1R 8 (TIR8), is a single immunoglobulin (Ig) associated receptor.[25] Unlike IL-18, IL-37 binding to IL-18Rα does not recruit IL-18Rβ chains to form functionally active ternary complexes to induce inflammatory cytokine production. Instead, IL-37 forms a complex with IL-1R8, IL-37/IL-18Rα/IL-1R8, that decoys myeloid differentiation factor 88 (MyD88) and restricts signaling downstream of the IL-1 family and TLR.[10,26,27] IL-18Rα and IL-1R8, as receptor chains of IL-37, has been detected in CD4+ T cells, DCs, innate lymphoid tissue cells, and different airway epithelial cells.[28] In addition, the expression of IL-1R8 has been found in monocytes, B lymphocytes, and NK cells.[29] IL-37 exerted anti-inflammatory effects via IL-1R8 by inhibiting the p38, ERK, JNK NF-κB, Fyn, MAPK, TAK1, and mTOR activation, and activating anti-inflammation pathways including AMPK, PTEN, Mer, STAT3, and p62. In IL-37 transgenic mouse (IL-37tg mouse) models, IL-1R8 deficiency causes mice to be susceptible to endotoxemia, and the anti-inflammatory activity of IL-37 is impaired by silencing either IL-1R8 or IL-18Rα.[30] As a result, the anti-inflammatory effect of IL-37 is mediated by two mechanisms, binding to IL-18Rα/SIGIRR and based on Smad3.

IL-18 binding protein (IL-18BP) can combine with IL-18 outside the cell to remove the latter, preventing IL-18 from binding to receptors and exerting anti-inflammatory effects.[31] However, excess IL-18BP combines with IL-37, reducing the anti-inflammatory activity of IL-18BP and IL-37. This action may explain why the anti-inflammatory effect of IL-18BP is weakened or even disappears when its level is increased.[26] Therefore, the anti-inflammatory effects of IL-37 are affected by IL-18Rα, IL-1R8, and IL-18BP (►**Fig. 1**).

Liver Protection by IL-37 in Different Liver Diseases

IL-37 Can Prevent Viral Hepatitis
Inflammation caused by viral infections, such as HIV-1, viral myocarditis, hepatitis B virus (HBV), and hepatitis C virus
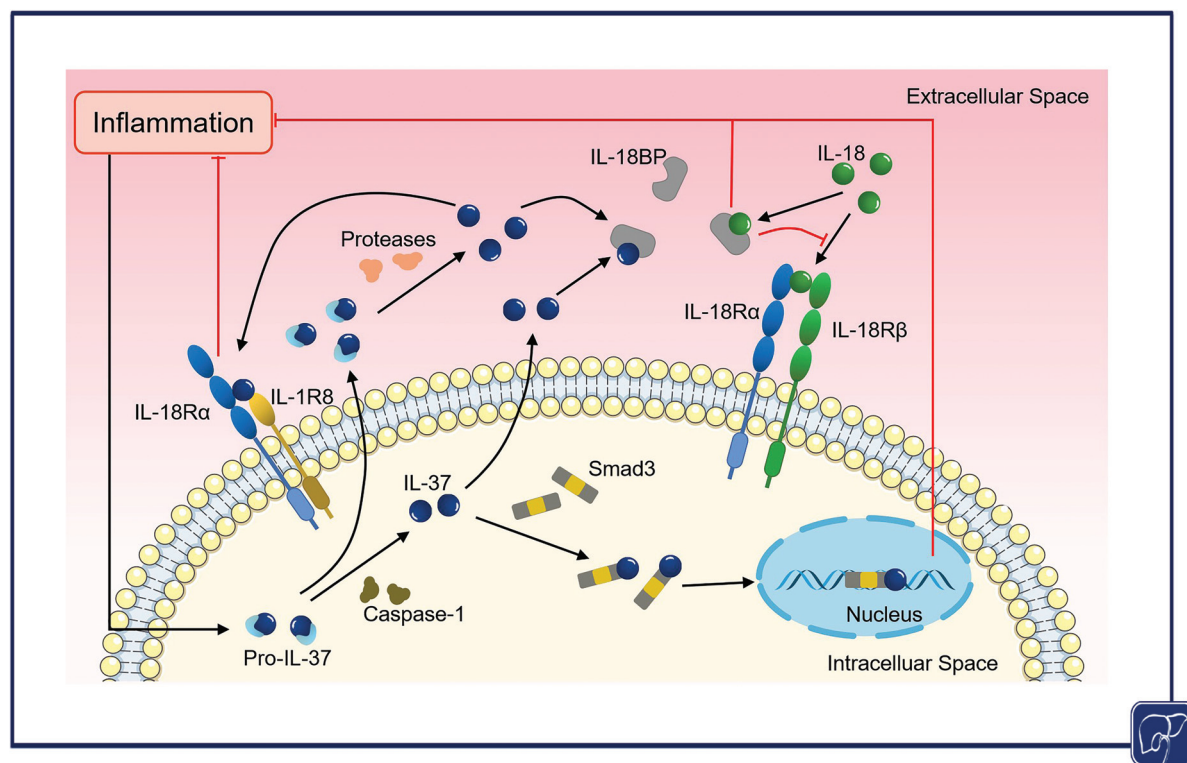
Fig. 1 IL-37 exerts dual anti-inflammatory activities inside and outside cells. In terms of the intracellular activity of IL-37, inflammation stimulates the production of intracellular IL-37 precursor and triggers the activation of caspase-1. Caspase-1 cleaves the IL-37 precursor to form mature IL-37. Most of IL-37 is secreted extracellularly, and a small portion binds to phosphorylated Smad3 and induces their translocation to the nucleus to regulate the expression of inflammatory genes. In terms of the extracellular activity of IL-37, cellular proteases cleave the IL-37 precursor extracellularly. Mature IL-37 binds to IL-18Rα and recruits IL-1R8 to form a complex that exerts the extracellular function of IL-37. IL-18 binds to IL-18Rα and IL-18Rβ extracellularly to induce inflammation, and IL-18BP can bind to IL-18 to inhibit this process, thereby inhibiting inflammation. However, excessive IL-18BP binding to IL-37 reduces the anti-inflammatory activity of IL-37 and IL-18BP.

(HCV), can be inhibited by IL-37.[32] Among these, HBV and HCV are the most common causes of viral hepatitis.

HBV, a hepatotropic noncytopathic small, enveloped DNA virus, escapes the recognition and attack of the immune system in various ways and uses "stealth" in the body, leading to chronic infection of liver cells. Currently, the escape mechanism of this virus remains unclear.[33] HBV-related antigens continue to stimulate the body, and chronic infection causes dilemmas in the immune system: adaptive immunity cannot be triggered rapidly and appropriately, or a state of depletion is induced, leading to an imbalance in the anti-HBV-specific immune response.[34] Cytokines, which are important components of the immune system, undergo tremendous disturbances and participate in the depletion of the anti-HBV immune response. Owing to its location and anatomical characteristics, the liver has a specific tolerance for the intestinal excretion of pathogens and antigens, and is thus protected from severe immune-mediated damage,[35] which also makes the invisible HBV more rampant in the liver, making the damaged adaptive response more difficult to manage.

The immune response of chronic HBV patients with HBeAg seroconversion may be influenced by IL-37[36]; however, the effect of IL-37 on the pathogenesis of HBV remains unclear. Through the evaluation of various cytokines, they

were found to have predictive value for disease progression control and as immunotherapy targets.[37] IL-37 can downregulate proinflammatory factors in liver injury, such as TNF-α, IL-12, IL-6, and IL-17, and upregulate anti-inflammatory factors, such as IL-4 and IL-10.[38,39] IL-4,[40,41] IL-6,[42–44] and IL-17[45] inhibit HBV replication and transcription. IL-12 not only promotes cellular immunity, but its combination with other therapies is also beneficial for HBV clearance.[46–49] T cell populations producing HBV-specific TNF-α may aggravate liver injury in patients with hepatitis B.[50] By regulating these factors, IL-37 may contribute to HBV infection. During HBV infection of primary human hepatocytes, the levels of the transforming growth factor-β (TGF-β) protein family in the supernatant are significantly reduced.[51] IL-37 can bind to Smad3, the downstream pathway target of TGF-β, to block the TGF-β pathway to exert anti-inflammatory effect; however, in HCC, IL-37 can also promote the release of TGF-β to inhibit tumor signal transduction.[52] Therefore, IL-37 may also play a role in HBV through TGF-β. IL-37 stimulation did not affect the secretion of proinflammatory cytokines in CD8+ T cells in patients with acute and chronic hepatitis B or the mRNA expression of programmed death-1 (PD-1) and cytotoxic T lymphocyte-associated protein-4 (CTLA-4). However, IL-37 inhibits the cytotoxicity of CD8+ T cells induced by HBV peptides through intercellular contact. Therefore, the

downregulation of IL-37 in acute HBV infection may be related to increased toxicity of CD8+ T cells and liver injury.[53] Single nucleotide polymorphisms (SNPs) in IL-37 have a protective effect against HBV clearance, and mutations in this gene may be related to the risk of HBV infection, by which infection with HBV and progression of HBV-mediated liver disease can be predicted.[54]

HCV is an ssRNA virus belonging to the Flaviviridae family. The incidence of hepatitis C is lower than that of hepatitis B; however, it can also lead to liver fibrosis, cirrhosis, and HCC.[55] In chronic HCV infection, the interaction between the virus and host immune factors results in an immune system imbalance that prevents the immune response from clearing the HCV infection, leading to persistent liver inflammation that can lead to cirrhosis, liver failure, or HCC.[56] Cytokines play crucial roles in the immune response against HCV infection. Single-cytokine therapy has been demonstrated to be effective against HCV infection[57]; however, no study has examined the effect of treatment with IL-37 on HCV infection. Although plasma IL-37 expression levels were found to be elevated in patients with HCV before treatment with IFN-α and ribavirin (RBV), these levels were decreased in patients with sustained virogenic response (SVR) and non-SVR after combination therapy.[58]

Although the mechanism underlying the direct effect of IL-37 on viral hepatitis remains unclear, the expression level of IL-37 varies before and after the onset of viral hepatitis, and may indirectly act on viral hepatitis by regulating cytokines related to HBV and HCV. Therefore, whether IL-37 can be used as a predictive risk factor for viral hepatitis, and whether it can directly affect viral hepatitis, requires further investigation.

IL-37 Attenuates Liver Injury

Alcohol, drugs, radiation, and ischemia/reperfusion can cause damage to the liver. Liver cell injury can activate inflammatory cells (macrophages and neutrophils) and release proinflammatory factors that aggravate the injury. IL-37, the only anti-inflammatory cytokine in the IL-1 family, is involved in the activation of inflammatory cells during liver injury caused by various factors. Here, we opted to review evidence of the involvement of IL-37 in the activation of inflammatory cells (mainly macrophages and neutrophils) induced by alcohol, drugs, and ischemia/reperfusion injury in the liver.

As an important cellular component of the liver, macrophages have become indispensable participants in maintaining liver homeostasis and in the injury and repair processes of acute and chronic liver diseases. Kupffer cells (KCs) can be activated after the liver is stimulated by injury through sensing disturbances in homeostasis. Activated KCs interact with the hepatic cell population to release chemokines which recruit circulating leukocytes, including monocytes, which subsequently differentiate into monocyte-derived macrophages in the liver.[59] Inflammation of the liver can be induced by KCs by releasing proinflammatory cytokines, such as TNF-α, IL-6, and IL-17.[60,61] In liver ischemia/reperfusion injury, following treatment with IL-37, the production of inflammatory mediators,

such as TNF-α, KCs, and macrophage inflammatory protein-2 (MIP-2), decreased, and the activities of several kinases, such as p38 MAPK and c-JUN, were downregulated in hepatocytes and KCs during the inflammatory response.[38] IL-37 was also found to inhibit KC infiltration after acetaminophen (APAP) injection.[61] In autoimmune hepatitis (AIH) and Concanavalin A (ConA)-induced T-cell–dependent liver injury model, the innate immune inhibitor, IL-37, directly inhibits the production of IL-1β/IL-12 by M1 macrophages to attenuate the Th1 response. This action facilitates Th2 responses and releases cytokines to promote the activation of hepatic macrophages toward M2, which not only further inhibits the expression of IL-1β and IL-12 but also increases the expression of IL-10 and IL-1Ra, thereby more effectively inhibiting the production of IFN-α by T cells in the liver. Simultaneously, the inhibition of M1 macrophage activation and increased M2 activation result in the potent suppression of TNF-α production in the liver.[39] However, whether AIH or ConA-induced liver injury model is employed, a long time is taken by IL-37 to trigger this regulatory network, which may be related to the flaky distribution of transgenic IL-37 in the liver.[23,39] Transfection of IL-37 into endometrial regenerative cells (ERCs) was found to result in the overexpression of IL-37, which further suppressed the production of CD4+ T cells and M1 macrophages in Con A-induced liver injury and promoted the induction of Tregs.[62] The enhancement of this effect may be related to the inhibition of M1 polarization by IL-37 through the inhibition of the nuclear factor-κB (NF-κB) and Notch1 signaling pathways.[63]

Neutrophils are a type of myeloid leukocytes, accounting for approximately 50 to 70% of circulating leukocytes, and have multiple functions in immune defense, including the production of reactive oxygen species (ROS), bactericidal proteins, neutrophil extracellular traps (NETs), and proinflammatory media.[64,65] Abnormal accumulation of neutrophils and excessive release of bactericidal compounds can damage human organs and tissues. Various liver diseases, such as NAFLD, alcoholic liver disease (ALD), and liver injury induced by factors, such as hepatic ischemia/reperfusion, endotoxins, and drugs, have been linked to neutrophil-mediated liver injury.[65] During activation, ROS production by neutrophils is called respiratory burst, and myeloperoxidase is the enzyme that plays a key role in this process.[66] The MPO activity was measured to indirectly assess the ability of neutrophils to migrate to mouse hepatocytes in a liver injury model. Neutrophil activation and respiratory bursts were reduced in liver injury models treated with IL-37,[38,61] suggesting that IL-37 can reduce the recruitment and activation of liver injury neutrophils (►**Fig. 2**).

Long-term alcohol consumption increases the risk of ALD, which can lead to liver fibrosis and cirrhosis, and even HCC. The pathogenesis of ALD includes hepatic steatosis, oxidative stress, toxicity mediated by ethanol metabolites, and elevation of proinflammatory cytokines and chemokines.[67] IL-37 plays a role in the suppression of inflammation in various disease models; however, in the ALD model, the sensitivity of IL-37tg mice to ALD was not found to differ from that of the wild-type control (alcohol-fed). This result may be because ethanol inhibits the expression of IL-37 in the liver tissue,
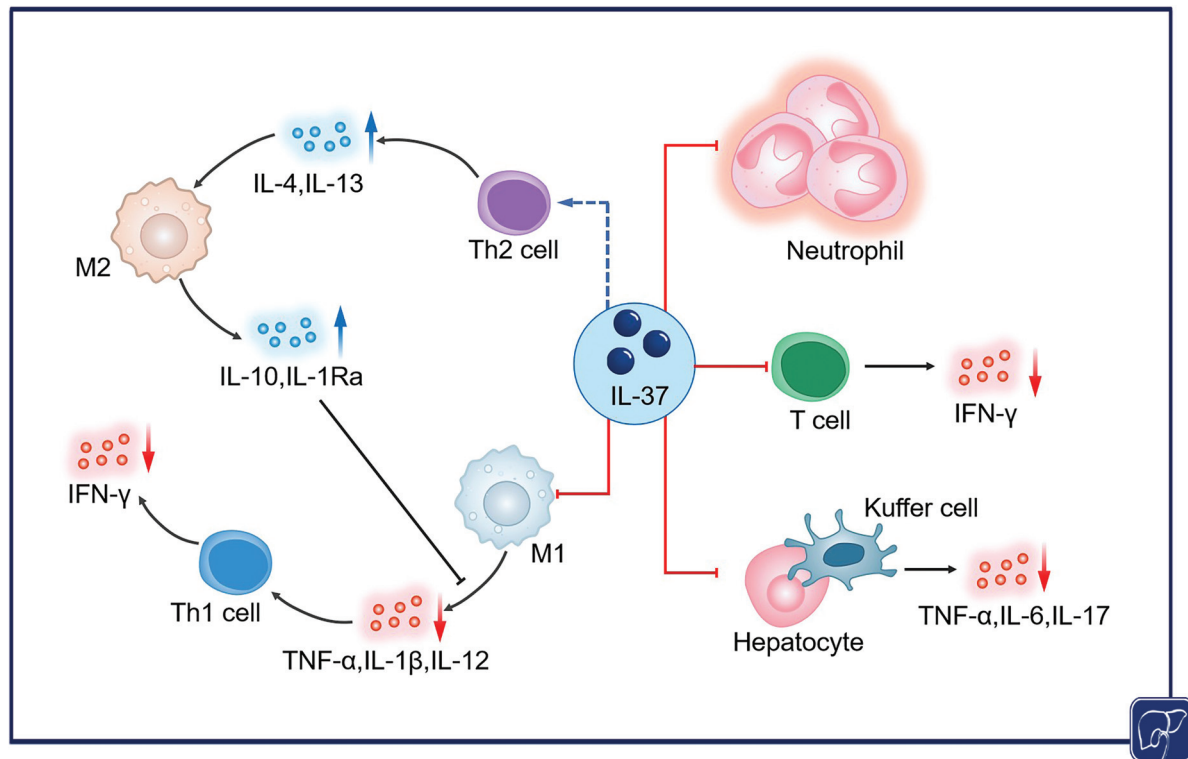
Fig. 2 Role of IL-37 in liver injury. IL-37 inhibits the secretion of IL-1β/IL-12 from M1 macrophages in liver injury to attenuate the Th1 response and decreases the secretion of IFN-γ, which indirectly promotes the Th2 cell response and secretes cytokines to promote hepatic macrophages to M2 type polarization and IL-10/1L-1Rα secretion. IL-37 also inhibits the recruitment of neutrophils, secretion of TNF-α/IL-6/IL-17 by hepatocytes and KCs, and secretion of IFN-γ by T cells during liver injury.

whereas recombinant IL-37 (rIL-37) treatment can improve liver inflammation and protect against steatosis in experimental ALD to a certain extent, which may be related to susceptibility to ALD. Of note, the mechanism by which ethanol inhibits IL-37 expression remains unclear.[68] So far, there are few clinical studies on the changes of IL-37 in liver injury. Compared with NAFLD patients, alcoholic steatohepatitis (ASH) patients exhibited reduced IL-37 expression. However, the author did not investigate IL-37 levels between ASH patients and healthy patients. When the liver is damaged, inflammation occurs frequently. Therefore, the expression of IL-37, as an anti-inflammatory factor, will increase during the process of liver injury to play an anti-inflammatory role.

In general, IL-37 inhibits the activation and infiltration of inflammatory cells during liver injury, reduces the production of proinflammatory factors, and stimulates the release of anti-inflammatory factors to attenuate the degree of liver injury. Macrophages exhibit strong plasticity and can be polarized into two phenotypes, M1 and M2, depending on the environment. When IL-37 is used to treat liver injury, it inhibits the polarization of macrophages to M1 type and promotes the activation of M2. Although M2 macrophages inhibit inflammation, they exert tumor-promoting effects. Therefore, whether IL-37 enhances cancer risk by promoting M2 polarization during liver injury requires further investigation.

IL-37 Targets Liver Fibrosis

Fibrosis is the internal response to chronic injury that preserves organ integrity during widespread necrosis or apoptosis.[69] Liver fibrosis is generally caused by chronic liver disease, and its main drivers are the activation of hepatic stellate cells (HSCs) and accumulation of extracellular matrix proteins.[70] Liver fibrosis can be caused by a variety of factors, such as HBV or HCV infection, alcoholism, NAFLD, and autoimmune liver disease. Long-term liver damage can lead to fibrosis and eventually cirrhosis.[70]

Liver injury promotes the activation of KCs and secretes TGF-β and platelet-derived growth factor (PDGF). PDGF is the main mitogen that activates HSCs, regulates the synthesis of collagen in HSCs, and promotes liver fibrosis. TGF-β is a core cytokine secreted by KCs, which can regulate the transition of HSCs toward myofibroblast-like cells, stimulate extracellular matrix (ECM) protein synthesis, and inhibit its degradation.[71,72] TGF-β and PDGF have been found to mediate Smad2/3 phosphorylation in HSCs through c-Jun NH2-terminal kinase (JNK), and transmit signals at the junctional region to promote HSC migration.[73] JNK may play a direct profibrotic role by promoting PDGF, TGF-β and angiotensin II–induced proliferation, α-SMA expression, and/or collagen production.[74] In addition to promoting liver fibrosis through the activin receptor-like kinase 5 (ALK5)-Smad3 pathway, TGF-β promotes fibrosis by activating another type I receptor pathway in HSC, the AlK-1-induced
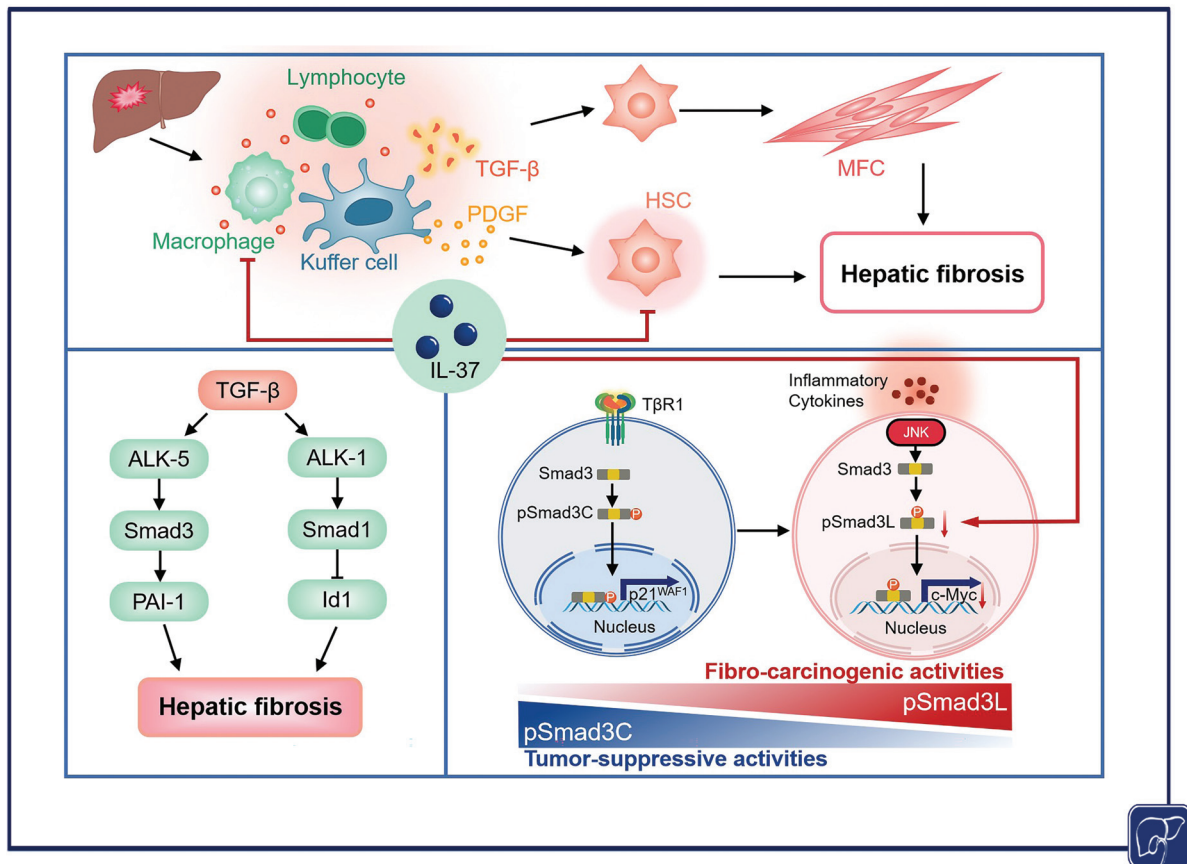
Fig. 3 IL-37 inhibits liver fibrosis. Liver injury stimulates lymphocyte infiltration, and macrophages and KCs release proinflammatory and profibrotic cytokines. KCs secrete PDGF and TGF-β. PDGF activates HSCs and regulates collagen synthesis. TGF-β regulates the transition from HSC to MFC, stimulates ECM protein synthesis, and promotes the occurrence of liver fibrosis. TGF-β promotes liver fibrosis via two pathways: (1) TGF-β activates the ALK5-Smad3 pathway to promote PAI-1 expression; (2) TGF-β activates ALK-1 to induce Smad1-mediated differentiation and inhibit Id1 expression. Under normal physiological conditions, after TβR1-mediated COOH-terminal phosphorylation of Smad3, pSmad3C translocates to the nucleus, activates p21[WIF1] gene transcription, and inhibits tumor activity. Under chronic inflammation and liver fibrosis, Smad3 in the cytoplasm undergoes linker phosphorylation, and pSmad3L translocates to the nucleus and activates the transcription of c-Myc gene, enhancing the oncogenic activity of fibers. IL-37 downregulates the c-Myc gene by co-localization with pSmad3L, directly targets the fibrosis pathway, and restricts cytokine release from lymphocytes, macrophages, and KCs to inhibit inflammation and HSC activation. HSC, hepatic stellate cell; MFC, myofibroblast-like cell; TGF, transforming growth factor.

Smad1 pathway, which mediates the expression of differentiation inhibitor 1 (Id1).[75]

As mentioned previously, the intracellular function of IL-37 is dependent on Smad3, a downstream kinase in the TGF-β pathway.[11] Smad proteins contain an intermediate junction region that connects the Mad homology domains, which transmit signals from the TGF-β type I receptor (TβRI) to the nucleus. Smad3 is differentially phosphorylated by membrane-bound cytoplasmic and nuclear protein kinases to generate C-terminally phosphorylated Smad3 (pSmad3C), linker-phosphorylated Smad3 (pSmad3L), and both linker and C-terminally phosphorylated Smad3 (pSmad3L/C).[76] JNK-dependent pSmad3L dominates and has profibrotic properties.[77] Intracellular IL-37 has been demonstrated to regulate liver fibrosis in two distinct ways: (1) by co-localizing with pSmad3L in cholangiocytes and hepatocytes, directly targeting fibrotic pathways, and (2) by restricting infiltrating lymphocytes, macrophages, and KCs to release proinflammatory and

profibrotic cytokines to downregulate liver inflammation and subsequent HSC activation.[78,79] Therefore, IL-37-dependent mechanisms can be targeted to treat inflammatory and fibrotic liver diseases (►**Fig. 3**).

IL-37 Inhibits HCC

A long-term accumulation of gene mutations leads to HCC, the most common form of primary liver cancer. According to the 2020 Global Cancer Statistics, primary HCC is currently the sixth most common cancer and third leading cause of cancer-related deaths.[80] HCC is extremely aggressive and has a low survival rate; its risk factors include chronic HBV and HCV infections, alcohol-induced cirrhosis, fatty liver disease, and obesity.[81] Important advances in HCC biology have revealed that activation or dysregulation of multiple molecular signaling pathways, such as the IL-6/JAK/STAT3 signaling pathway, TGF-β signaling pathway, PI3K/AKT/mTOR signaling pathway, can promote HCC.[82–85] Cytokines are

important components of the immune system and can affect the development of HCC.[86] The occurrence and development mechanisms of HCC are complicated owing to the long-term accumulation of gene mutations.[3] However, the mechanism of action of IL-37 in HCC remains unclear. Compared with healthy liver tissues and adjacent nontumor tissues, tumor tissues express less IL-37, which is associated with poor tumor progression and prognosis.[87] In addition to inhibiting angiogenesis in tumors by reducing the expression of proangiogenic factors, such as matrix metalloproteinase 2 (MMP2) and vascular endothelial growth factor (VEGF), in the tumor microenvironment,[88] IL-37 can also inhibit tumors by inhibiting the activation of tumor-associated signaling pathways.[89]

IL-37 Regulates the IL-6/JAK/STAT3 Signaling Pathway to Inhibit the Growth of HCC Cells

The IL-6/JAK/STAT3 signaling pathway is aberrantly overexpressed in many human cancers and is associated with cancer initiation and progression.[90] Based on increasing studies, IL-6 plays a proinflammatory role in the tumor microenvironment, mediates STAT3 activation, drives liver cell repair and replication, and promotes liver carcinogenesis and invasion of HCC cells.[91–93] IL-6 is highly expressed in HCC tissues, is transported in the serum, and is related to the stage, severity, and prognosis of HCC.[94]

IL-37 may inhibit epithelial mesenchymal transformation (EMT)-mediated tumor migration and invasion in HCC by inhibiting IL-6/STAT3 pathway signaling, increasing E-cadherin, and inhibiting vimentin expression.[95] Zhang et al recombinantly expressed the IL-37 gene in vaccinia virus (VV), which can infect, replicate, and lyse tumor cells. Based on their findings, VV-IL-37 exhibits strong tumor-specific cytotoxicity by inhibiting STAT3 phosphorylation, indicating that VV-IL-37 could serve as a potential anticancer drug.[96,97] IL-37 can also promote the polarization of M2 macrophages into M1 macrophages by inhibiting this signaling pathway to inhibit the growth of HCC cells.[98] However, as previously revealed, IL-37 directly inhibits the activation of M1 macrophages in hepatitis models,[39] whereas the opposite is true for the regulation of M1 macrophages in hepatocellular carcinogenesis. IL-37 inhibits M1 macrophage polarization in atherosclerosis but promotes this polarization in colon cancer. Researchers have hypothesized that this differential regulation may be due to the large number of bacteria in the gut and the lack of pathogens in the cardiovascular system.[99] The differential regulation of IL-37 in M1 macrophages in liver inflammation and HCC may be due to the dominance of M1 macrophages (which can promote inflammation) in excessive inflammation. Notably, IL-37 regulates excessive inflammation to inhibit inflammation. In the later stage of tumor, M2 macrophages (which can promote tumor growth) dominate, and IL-37 promotes the inflammation of tumor to play an antitumor role. However, in the early stages of tumorigenesis, macrophages are primarily polarized into the M1 type to clear tumor cells. As the mechanism whereby IL-37 regulates the macrophage phenotype is unknown, further studies are warranted.

Combination of IL-37 and Smad3 Changes TGF-β Signaling in HCC

The multifunctional cytokine TGF-β plays an important role in tumor formation, progression, and metastasis. In the classical pathway of TGF-β signaling, the phosphorylation and activation of Smad2 and Smad3 is caused by the binding of TGF-β to TGF-β RII to trigger the phosphorylation of TGF-β RI. Activated Smad2/3 binds to Smad4 to form the Smad transcription complex, which then translocates to the nucleus to activate the transcription of downstream genes.[100,101] TGF-β/Smad signaling homeostasis in vivo is regulated by feedback loops, such as the negative feedback loop between TGF-β and Smad7. Activated by Smad2/3, Smad7 inhibits TGF-β/Smad signaling by binding to TGF-β RI/II.[102]

In the liver, hepatic sinusoidal endothelial cells and HSCs are the main producers of TGF-β. TGF-β activity has been demonstrated to be key to HCC pathogenesis, including activation of tumor-associated fibroblasts (CAFs), tumor-associated macrophages, and other tumor-associated cells.[103–105] In fact, TGF-β plays a dual role in HCC.[106,107] An earlier comparative functional genomics study found that two distinct groups of TGF-β responsive genes may exist.[108] The complex role of TGF-β in the progression from inflammation to fibrosis to HCC was summarized by Gough et al.[109] The cytostatic factor TGF-β induces apoptosis and inhibits cell proliferation in normal hepatocytes and in most early-stage HCC.[107,110] In advanced HCC, serum levels of TGF-β were found to be significantly elevated and correlated with poor tumor prognosis.[111] TGF-β can be activated by the tyrosine kinase receptor, Axl, to induce the expression of MMP8 and myocyte enhancer factor-2 (MEF2) to promote the EMT and malignant progression of HCC cells.[112–114]

In the process of human hepatocellular carcinogenesis, pSmad3C in the differential phosphorylation of Smad3 is transferred to the nucleus to activate p21^{WAF1} gene transcription and inhibit cell growth, whereas pSmad3L stimulates cell growth by upregulating c-Myc transcription.[76] According to some researchers, the upregulation of IL-37 promotes the activation of G2/M phase arrest in HCC cells to inhibit tumor growth. The binding domain of the IL-37–Smad3 complex shields the phosphorylation site of pSmad3L, directly targeting the JNK/pSmad3L/c-Myc signal to downregulate the expression of the oncogene, c-Myc. IL-37 also promotes the secretion of TGF-β and inhibits the secretion of tumorigenic factors, such as IL-16, IL-8, and MMP2, thereby positively feeding back the regulation of TGF-β/Smad3 signal transduction. Therefore, IL-37 promotes Smad3 phosphorylated isoform signaling switches from JNK/pSmad3L/c-Myc oncogenic signaling to pSmad3-C/p21 tumor-suppressive signaling.[52] Although the effect of IL-37 on the phosphorylation pattern of Smad3 provides a new idea for the study of the mechanism of HCC, TGF-β has a bidirectional effect in HCC. IL-37 also promotes the release of TGF-β in HCC to enhance the transduction of pSmad3-C/p21 tumor suppressor signals.[52] Therefore, the role of IL-37 in the context of high expression in advanced HCC remains unclear.

IL-37 Inhibits the PI3K/AKT/mTOR Signaling Pathway to Induce Autophagy and Apoptosis of HCC Cells

In the PI3K/AKT/mTOR signaling pathway, PI3K binds to various cytokines and growth factor receptors to activate AKT, and the activated AKT activates several proteins via phosphorylation and regulates various key cellular activities. The mammalian target of rapamycin (mTOR) protein subfamily is an important downstream effector of AKT.[85] The mTOR signaling pathway is aberrantly expressed in HCC and is strongly activated at the tumor margin, thereby enhancing the proliferation and spreading ability of HCC.[115] In addition to cell proliferation, apoptosis, and angiogenesis, this pathway also participates in autophagy.

Autophagy, a lysosomal degradation pathway, plays a complex role in the development of tumors and can inhibit or promote the occurrence of tumors, which is also the case for HCC.[116,117] Qu et al confirmed the inhibitory effect of autophagy on HCC by knocking out key autophagy-related genes in mice.[118] However, in the advanced stages of cancer, when the tumor is under extremely stressful conditions of hypoxia and nutrient deprivation, autophagy can enhance the survival of tumor cells in the hypoxic zone of the tumor[119] and provide metabolic substrates by recycling intracellular components to satisfy the high metabolic and energy demands of tumor proliferation.[120,121] The development of benign liver tumors into malignant HCC is inseparable from the role of autophagy, which promotes the occurrence of HCC by maintaining liver cancer stem cells.[122] mTOR is composed of two complexes: mTORC1 and mTORC2. Activated mTORC1 plays a key role in the phosphorylation of autophagy-related proteins and can inhibit autophagy, which is enhanced when inhibited under various stress conditions, such as starvation or organelle damage. However, the role of mTORC1 in autophagy remains unclear.[123]

IL-37 induces autophagy and apoptosis of HCC cells by inhibiting the PI3K/AKT/mTOR signaling pathway while simultaneously inhibiting the proliferation of HCC cells.[124] Although IL-37 regulates the autophagy pathway by downregulating mTOR expression, its specific targets in the autophagy-related pathways of HCC cells are unclear. Accordingly, more in-depth research is needed.

IL-37 and Tumor Immune Response

The liver has a complex microenvironment under normal physiological conditions. Due to its location, the intestinal tract not only transports nutrients to the liver but also continuously transports various antigens, which gives the liver specific immune tolerance to avoid severe immune impairment.[125] However, chronic inflammatory liver disease can disturb this immune tolerance network, thereby promoting the development of liver tumors. In recent years, immunotherapy targeting the immune system of HCC patients rather than tumor cells or tissues has led to remarkable changes to the treatment of HCC, such as single drug therapy and combination therapy targeting CTLA-4, PD-1, other immune checkpoints, chimeric antigen receptor (CAR) T cells, and other immune cell therapies to improve the immunosuppressive environment in the tumor microenvironment.[126–128] In HCC, the depletion and functional failure of DCs, T cells, and other immune cells provide a favorable growth environment for HCC, and tumor-associated macrophages, MDSC, and other immunosuppressive cells in the tumor microenvironment promote the escape of HCC cells.[125] Therefore, restoring immune homeostasis in the liver microenvironment and enhancing liver immunity are potential therapeutic strategies for the treatment of HCC.

Numerous studies have shown that IL-37 can inhibit the development of HCC by affecting immune and immunosuppressive cells in the HCC microenvironment and has the therapeutic potential to restore homeostasis of the liver immune system. Immune cells called NK cells play a crucial role in preventing tumors from growing.[129] Low expression of IL-37 in HCC cells weakens the ability of NK cells to be recruited to tumor tissues, resulting in a defect in the antitumor immune microenvironment. In contrast, the overexpression of IL-37 in HCC mice significantly slowed down tumor growth, and more NK cells were recruited to the tumor tissue to play a tumor-killing role.[87] In contrast to the results of this study, Gao et al found that the antitumor immunity of IL-37 depended on T cells and B cells rather than on NK cells in their study on fibrosarcoma[18]; this dependency may be the reason for the different types of cancer studied. High expression of IL-37 can enhance the recruitment and activation of DCs in HCC, thereby inducing cytotoxic T lymphocytes (CTLs) to exert antitumor effects.[130] IL-37 also promotes the polarization of M2 macrophages with tumor-promoting effects into M1 macrophages with antitumor effects to inhibit the growth of HCC cells.[98]

IL-37 promotes tumor immunity and inhibits HCC development during HCC progression. However, IL-37 can also play an immunosuppressive role in other tumors by promoting tumor occurrence and immune escape, which are conducive to the occurrence and development of tumors. Huo et al reported that serum IL-37 is elevated in patients with epithelial ovarian cancer and is associated with poor overall survival and shortened progression-free survival.[131] More studies have found that IL-37 in the serum of colorectal cancer patients is negatively correlated with CD8+ T cell infiltration, which proves that IL-37 plays an oncogenic role in colorectal cancer by mediating T cell dysfunction; however, the TGF-β/Smad3 signaling pathway is not involved in this process.[132] Analysis of immune cells in the blood samples of patients with melanoma revealed the elevation of IL-37 in Tregs and a series of data showed that IL-37 has a potential immunosuppressive effect on the occurrence of melanoma. Highly elevated IL-37 may serve as a biomarker for tumor-induced immunosuppression in certain lymphocyte populations[133] (►Table 1).

IL-37 from immune cells has an immunosuppressive effect on the tumor microenvironment and promotes tumor progression. As indicated above, IL-37 plays a protective role in cancer by inhibiting multiple signaling pathways and enhancing its antitumor effects. This protective effect may

Table 1 Effects of IL-37 in liver disease

Model of disease		Effects of IL-37	Ref.
Liver injury	I/R	• Activation of neutrophil (↓) • Release of TNF-α, MIP-2, and KCs (↓)	38
	APAP	• Expression of TNF-α, IL-6, IL-17, and NF-κB (↓) • Numbers of KCs (↓) • Protein expression of NF-κB p65 (↓)	61
	ConA	• Levels of ALT and AST (↓) • Proportion of M1 macrophages and CD4$^+$ T cells (↓) • Proportion of Tregs (↑) • Levels of IL-12, IFN-γ (↓) • Levels of TGF-β (↑)	62
	ConA, AIH	• Expression of IFN-γ, TNF-α, IL-1β, IL-12 (↓) • Expression of IL-4, IL-13, IL-10, IL-1Ra (↑) • Activation of M1 macrophages (↓) and M2 macrophages (↑) • Response of Th1 (↓) and Th2 (↑)	39
Liver fibrosis		• Expression of IL-6, TGF-β, Cxcl1 (↓) • Proinflammatory response of HSC and KCs (↓)	78
HCC		• Expression of MMP2, MMP9, VEGF (↓)	88
		• Numbers of NK cells (↑)	87
		• Expression of pSmad3L, c-myc (↓) • Expression of pSmad3C, p21, TGF-β (↑)	52
		• Expression of IL-6, pSTAT3 (Y705), N-cadherin, and vimentin (↓)	95
		• Activation of M1 macrophages (↑) and M2 macrophages (↓)	98
		• Release of IL-2, IL-12, IL-12p70, IFN-α, and IFN-γ (↑) • Numbers of DCs (↑)	130

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; APAP, acetaminophen; AST, aspartate aminotransferase; ConA, Concanavalin A; DCs, dendritic cells; HCC, hepatocellular carcinoma; HSC, hepatic stellate cells; I/R, ischemia/reperfusion; IFN-γ, interferon-γ; KCs, Kupffer cells; MIP-2, macrophage inflammatory protein-2; MMP2, matrix metallopeptidase 2; MMP9, matrix metallopeptidase 9; NK cells, natural killer cells; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; Tregs, regulatory T cells; VEGF, vascular endothelial cell growth factor.

be mediated through the transfection of IL-37 into cancer cells or animal models, enabling IL-37 to directly act on the tumor itself and inhibit HCC. However, there are few reports on the mechanism by which IL-37 acts indirectly on HCC through immune cells. Therefore, whether IL-37 from immune cells can promote HCC needs to be investigated in the later stages (►Fig. 4).

Conclusion and Prospects

IL-37 plays a protective role against hepatitis, liver injury, liver fibrosis, and HCC. IL-37 expression differs before and after the onset of viral hepatitis. Although there is no direct evidence to explain how IL-37 regulates the mechanism of viral hepatitis, IL-37 can affect the expression of cytokines related to viral hepatitis. Thus, we can search for the target of the effect of IL-37 on viral hepatitis from this aspect. As mentioned above, IL-37 differentially regulates macrophages before and after the occurrence of HCC. For example, IL-37 inhibits the polarization of M1-type macrophages in hepatitis and liver injury to inhibit the occurrence of inflammation and promotes the polarization of M2 to M1 in HCC to kill tumors. However, the mechanisms underlying differential regulation remain unclear. According to Zhang et al, IL-37 promotes M1 polarization in HCC by inhibiting the

IL-6/STAT3 pathway[98]; however, these researchers did not investigate whether activation of the IL-6/STAT3 pathway in hepatitis would affect the regulation of IL-37 in macrophages. Although the fundamental role of IL-37 in liver disease has not been well-studied, an effective cytokine therapy may benefit from the role played by IL-37 in innate and adaptive immunity.

To date, no clinical trials have proven the impact of IL-37 on the treatment of liver diseases; however, basic research has shown that the combined detection of IL-37 and HCC markers can improve the sensitivity and accuracy of HCC diagnosis and prognosis. Elevation of the tumor marker, serum α-fetoprotein (AFP), can predict the occurrence and development of HCC, and normal liver cells express very low levels of AFP.[134] By assessing the correlation between IL-37 and serum AFP in patients with HCC, the inhibition of IL-37 expression was found to be potentially related to an increase in AFP secretion in cancer cells; however, the expression of IL-37 in paracancerous tissues is unrelated to serum AFP.[135] HCC is difficult to diagnose owing to its strong concealment; therefore, the combination of AFP detection and cancerous IL-37 can improve the sensitivity and accuracy of HCC diagnosis and prognosis.[136] Thus, IL-37 may play an important role in the diagnosis and treatment of HCC. A research team pointed out that the combination of cytokine-induced
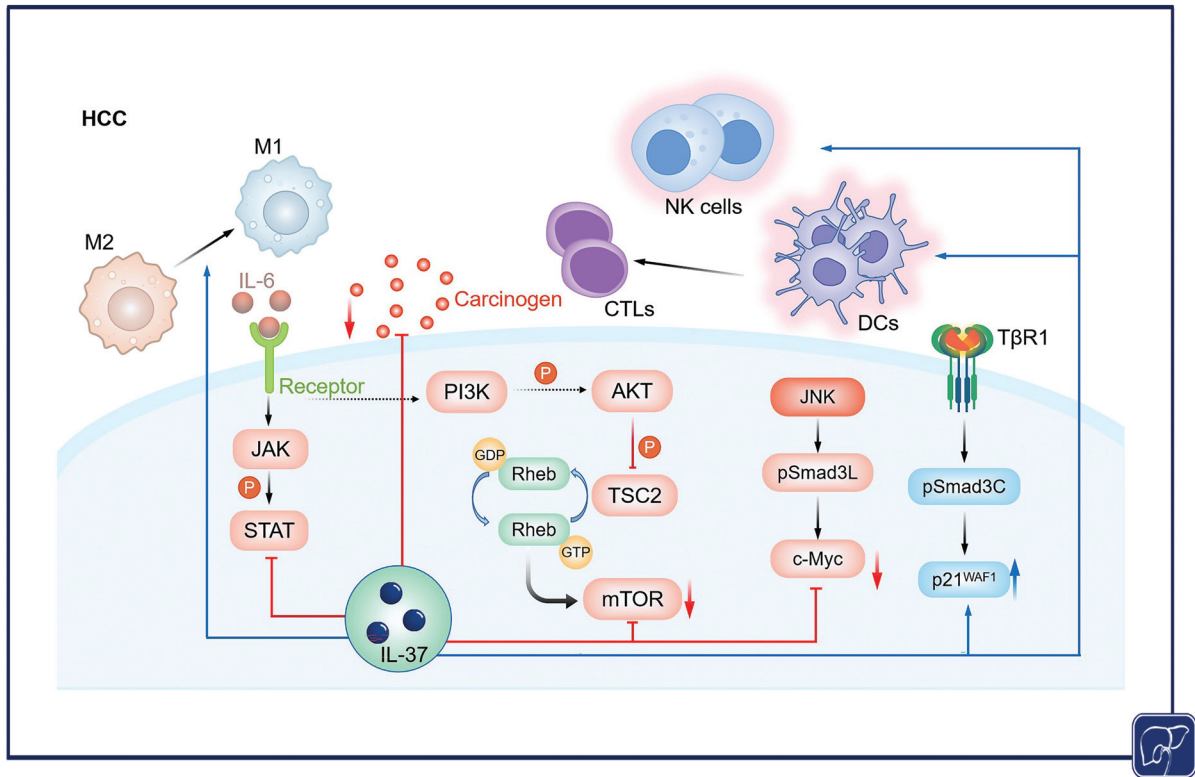
Fig. 4 IL-37 inhibits hepatocellular carcinoma (HCC). IL-37 regulates the IL-6/JAK/STAT3 signaling pathway to inhibit the growth of HCC, downregulates the PI3K/AKT/mTOR signaling pathway to induce autophagy and apoptosis of HCC cells, and converts TGF-β signaling in HCC from the JNK/pSmad3L/c-Myc oncogenic signal to the pSmad3C/p21 tumor suppressor signal. IL-37 also recruits NK cells into tumor tissues to kill tumors. IL-37 recruits and activates DCs and induces CTLs for tumor killing. IL-37 promotes the polarization of M2 macrophages into M1 macrophages, inhibits the production of oncogenic factors, and regulates the growth of HCC cells.

killer cell adoptive therapy and IL-12-expressing oncolytic adenovirus can exert an antitumor effect of "$1+1>2$" in HCC models.[137] IL-37, a new type of cytokine that naturally suppresses innate inflammation and immune response, can also be applied to this combination therapy to exert stronger and more effective antitumor activity. Existing studies have suggested that the combination of viral and IL-37 gene therapy vectors can provide the best gene therapy combination for HCC,[97,136] suggesting that IL-37 may be developed into a clinical adjuvant therapy for liver diseases in the future, and even as a promising approach to HCC.

Overall, IL-37 may not only be a valuable prognostic biomarker but also a potential candidate for gene therapy for liver diseases. However, the study on the mechanism of IL-37 on liver diseases is not entirely accurate; for example, there is no research on the mechanism of IL-37 on viral hepatitis. In addition, the expression of IL-37 receptor, which is used in clinics, will have several negative effects. To play a therapeutic role in the treatment of liver diseases, it is urgent to identify the specific receptor target of IL-37, or to determine whether the IL-37 gene can be modified to make it more specifically target abnormal cells in liver diseases, this will allow IL-37 to be more effectively applied in future clinical adjuvant therapy of liver diseases and improve the accuracy and sensitivity of liver disease diagnosis.

Authors' Contribution
B.J. and Y.Z. contributed to manuscript writing; Y.L., B.L., and T.P. conducted literature retrieval; and L.Y. and L.Q. revised the manuscript.

Conflict of Interest
None declared.

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