Tumor Immune Microenvironment

Subjects: Pathology

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Since the efficacy of ICIs depends on the tumor immune microenvironment, it is necessary to elucidate the immune environment of HCC to select appropriate ICIs.

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1. Introduction

1.1. Hepatocellular Carcinoma (HCC)

The incidence of liver cancer has increased worldwide, not only in East Asia but also in western Europe and the United States. Liver cancer currently ranks sixth in incidence rate and fourth in mortality rate of all cancers [1]. Hepatocellular carcinoma (HCC) is the most common subtype, accounting for more than 90% of all primary liver cancers [2]. Multiple etiological risk factors are associated with the incidence of HCC, including chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), alcohol abuse, non-alcoholic steatohepatitis (NASH), autoimmune liver disease, drug-induced liver injury, and aflatoxin exposure [2][3]. Despite great therapeutic advances, HCC has one of the worst prognoses with a 5-year survival rate of 15–38% in the United States [4][5] and Asia [6] due to late diagnosis, resistance to chemotherapy, and frequent recurrence and metastasis.

Treatment options such as surgical resection, radiofrequency ablation, and transarterial chemoembolization are effective for HCC localized in the liver, while systemic therapy with various drugs targeting the tumor microenvironment (TME) is available for unresectable HCC. Since sorafenib was first shown to prolong the survival of patients with unresectable HCC [7], systemic therapy with molecular-targeted agents (MTAs) has continued to evolve significantly as a useful therapeutic strategy for advanced HCC. Multikinase inhibitors such as sorafenib, lenvatinib, regorafenib, and cabozantinib, as well as the vascular endothelial growth factor (VEGF) inhibitor ramucirumab, have found widespread clinical applications [8][9][10][11]. In addition to MTAs, new therapeutic strategies such as cancer immunosuppressive therapy based on immune checkpoint inhibitors (ICIs) have progressed in recent years. For advanced HCC, the combination of ICIs and VEGF inhibitor has shown better results than sorafenib [12], and the combination of atezolizumab and bevacizumab (atezo+bev) is now positioned as the first-line therapy for patients with advanced HCC. Although systemic therapies for HCC have undergone a major paradigm shift, treatment for advanced HCC remains inadequate because of a lack of evidence associated with treatment resistance and prediction of treatment response. Since the efficacy of immunosuppressive therapy including ICIs depends on the tumor immune microenvironment, it is necessary to elucidate the immune environment of HCC to improving current core treatment strategies and prognosis for HCC patients.

1.2. Tumor Microenvironment (TME)

In addition to cancer cells, the TME includes innate and adaptive immune cells, stromal cells, endothelial cells, and cancer-associated fibroblasts. In the TME, immune cells such as macrophages infiltrate, fibroblasts proliferate, and angiogenesis is induced, and the TME is reportedly deeply associated with the formation, survival, and metastasis of tumor tissues [13]. Furthermore, the development of treatment approaches that target angiogenesis, adhesion, and infiltration of tumor cells in the TME is also in progress. Although previous studies have focused on adaptive immune cells due to the potent cytotoxicity of T lymphocytes in the context of cancer, current TME-targeted treatments have predominantly focused on the innate T cell immune responses, including checkpoint blockade and chimeric antigen receptor (CAR) T cell therapies. In the treatment of advanced HCC as well as other cancer types, immunotherapeutic approaches have increasingly focused on monoclonal antibodies against cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1), which lock the immune checkpoint inhibition pathways [14].

Therefore, exploring the immune microenvironment associated with these treatment strategies contributes to improved outcomes in patients with advanced HCC.

2. The Immune Microenvironment in Liver Inflammation and Fibrosis

During chronic liver injury caused by pathogens such as the hepatitis virus and parasites or by drugs, alcohol, and steatosis, both the systemic and local immune microenvironments regulate liver inflammation and fibrosis, leading to hepatocarcinogenesis. In addition, liver cirrhosis is also a state of immune dysfunction caused by the excessive activation of proinflammatory cytokines [15]. In patients with liver cirrhosis, the risk of bacterial infection and sepsis increases due to the abnormal gastrointestinal barrier permeability and bacterial translocation; therefore, it is useful to elucidate the immune microenvironment during chronic liver disease to improve prognosis [16].

2.1. Immune Regulation and Microenvironment in Liver Inflammation

Inflammatory mediators produced by liver-resident immune cells play pivotal roles in maintaining local liver and systemic homeostasis, and resident myeloid cells contribute to the maintenance of hepatic tolerance [17]. In response to bacterial endotoxins, KCs produce anti-inflammatory cytokines such as interleukin (IL)-10 and prostaglandins, which subsequently suppress the expression of co-stimulatory molecules on antigen-presenting cells, preventing CD4 + T cell activation. Compared with those in the spleen, hepatic myeloid DCs also produce significantly more IL-10 and suppress T cell activation [17][18]. In addition, myeloid-derived suppressor cells (MDSCs) produce immunosuppressive cytokines, including IL-10 and TGF-β, to maintain a tolerogenic liver environment.

The liver also plays a central role in the detection of and response to inflammatory signals. Cytokines produced by extra-hepatic immune cells are detected by hepatocytes in the hepatic blood flow following an increase in acute-phase protein production and synthesis of IL-6, which increases the acute systemic response [17]. Acute-phase proteins produced directly by hepatocytes promote systemic inflammatory responses, such as massive immune cell infiltration to the initial inflammatory site, while controlling the processes necessary to suppress excessive inflammation. These processes include suppression of neutrophil function by protease inhibitors, decreased TNF production, suppressive MDSC recruitment by amyloid A, control of bystander tissue damage by the inflammatory process, and enhancement of the repair process [19].

Liver-resident immune cell populations and inflammatory mediators such as IL-1α, TNF-α, and IL-6 are indispensable for liver regeneration after liver injury and control metabolism and xenobiotic detoxification. Reports demonstrating the importance of these inflammatory mediators in liver regeneration show that targeted IL-6 disruption caused hepatic regeneration disorder in mice. Its function recovered with a single dose of IL-6, and impaired liver generation was also observed in mice treated with antibodies targeting TNF-α [17].

2.2. Hepatic Immune Microenvironment with Progression to Fibrosis

Liver fibrosis is the final destination of pathological hepatic disorders in chronic liver diseases such as viral hepatitis, alcoholic liver disease, and liver steatosis. HSCs and KCs play important roles in the progression of liver inflammation to fibrosis. Several proinflammatory factors, including TGF-β and platelet-derived growth factor (PDGF), activate HSCs through TLR4, which then produce extracellular matrix proteins such as various subtypes of collagen [20]. TLR4 has been shown to play important roles in regulating liver damage, and mice with hepatocyte-deleted TLR4 were reportedly protected against chronic alcoholic liver disease and fatty liver [21]. In addition to TLR4, HSCs also express TLR3 and TLR9, which are related to liver fibrosis. The TLR3 ligand, polyinosinic-polycytidylic acid, activates NK cells through high expression of TNF-α-related apoptosis-inducing ligand (TRAIL) and induces cell death of activated HSCs, thus resulting in reduced severity of liver fibrosis [22]. TLR9 upregulates HSCs under the influence of host origin DNA from apoptotic DNA, enhancing liver fibrosis [23]. KCs also cause liver fibrosis through activation of TLRs by lipopolysaccharides, triggering the production of several cytokines, including TGF-β. Among other pattern recognition receptors, besides TLRs, only the NOD-like receptor (NLR) is associated with liver fibrosis progression. NLRs form bioactive protein complexes called inflammasomes, which produce proinflammatory cytokines such as IL-1β and IL-18, resulting in the activation of HSCs in chronic inflammatory liver diseases [24]. Recently, selective inflammasome inhibitors have been shown to exert proactive effects in cholestatic liver injury and liver fibrosis in a mouse model [25].

NK cells inhibit the progression of liver fibrosis by killing activated HSCs [26]; therefore, the association between NK cells and fibrosis in various liver diseases has been highlighted. In HCV-infected patients, the accumulation of NK cells with highly expressed NKp64 receptors, which showed potent cytotoxic activity and IFN-γ secretion, was inversely correlated to HCV-RNA levels and the degree of liver fibrosis [27]. In a mouse model of chronic alcohol consumption, induction of

TSC resistance to NK cell killing, desensitization of HSC resistance from NK toxicity, and inhibition of IFN-γ accelerated liver fibrosis [28]. Signal transduction and activation of transcription 1 (STAT1) signaling antagonize the effects of TGF produced by HSCs and negatively regulate fibrosis to support NK cytotoxicity [29].

NKT cells, which are mainly present in the liver, are also central immune players in the progression from liver inflammation to fibrosis; however, their function in fibrosis seems to be uncharacterized and influenced by various conditions [30]. In mice lacking mature NKT cells caused by disruption of the CD1d molecule, thioacetamide-induced hepatocellular inflammation and damage were ameliorated, and the profibrogenic response of tissue inhibitor of matrix metalloproteinase (TIMP) 1 was significantly reduced [31]. In a study of a fibrosis-induced model with carbon tetrachloride (CCl4), NKT cell-deficient mice were found to be more susceptible to CCl4-induced liver inflammation. Although strong activation of NKT cells by α-galactosyl ceramide accelerates CCl4-induced liver fibrosis, CCl4 administration induced only a slightly higher degree of liver fibrosis in NKT cell-deficient mice than that in control mice at 2 weeks but not 4 weeks after induction by CCl4. During chronic liver injury, NKT cells inhibit liver fibrosis in the early stage but may not affect the late stage due to the depletion of NKT cells [32].

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