

Review

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Mechanisms and immunotherapies of HBV- and NAFLD-related hepatocellular carcinoma

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Abstract

Hepatitis B virus (HBV) infection remains the most important risk factor for hepatocellular carcinoma (HCC) worldwide and nonalcoholic fatty liver disease (NAFLD) has developed as major etiology of chronic liver diseases, cirrhosis and eventually HCC in the last decades. Although nucleos(t)ide analogs are recommended as the first-line drug for patients with chronic hepatitis B, incomplete eradication of HBV serves as an obstacle for effective cure of chronic hepatitis B and even HCC. NAFLD refers to a spectrum of hepatic metabolic disorders, compromised with multi-system diseases. Considering the specificity of hepatocytes and enrichment of immune cells in liver, this review aims to summarize the mechanisms of direct pro-tumorigenesis to hepatocytes induced by HBV infection and abnormal lipid metabolism, and indirect oncogenic processes mediated by immune cells. We also discuss similarities and differences of immune cells between HBV- and NAFLD-HCC and finally focus on the novel immunotherapies concerning preclinical and clinical studies for liver cancer.

Keywords: Hepatitis B virus, nonalcoholic fatty liver disease, hepatocellular carcinoma, immune cells, immunotherapy

INTRODUCTION

Liver cancer is one of the most common causes of cancer-related deaths worldwide, with an estimated of 1.1 million new cases to be diagnosed in 10 years (GLOBOCAN, 2018). Hepatocellular carcinoma (HCC) accounts for nearly 80% of primary liver cancer and the occurrence is globally gender and geography biased. The estimated age-standardized incidence rates in men are mostly 3 times than that of women (13.9/1,000,000 vs. 4.9/1,000,000) in 2018. The high rates in Pacific Asia, West and Central Africa have persisted for decades, although declining trends have been observed in China and Japan in the past two



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decades due to therapy advancement and control of etiology. In contrast, incidence and mortality rates in North America have been increasing since 1978, for which the main reasons are cumulative emergence of new risk factors and lack of efficient therapy strategies.

HCC is prominently induced by chronic hepatitis B virus (HBV)/hepatitis C virus (HCV) infection, alcoholic or nonalcoholic fatty liver disease, aflatoxin intake and parasitic infection, the incidence of which are related with geographic variance. About 80% of HCC arises from persistent HBV infection in Asia-Pacific and Africa countries, while non-alcoholic fatty liver disease (NAFLD) is the major contributor in North America, followed by HCV infection. The direct-acting antiviral therapy with high rates of safety and sustained virologic response (> 95%), is considered as the contributing factor for eliminating HCV^[1,2], which helps decrease the pathogenesis of HCC. However, HBV-associated HCC remains to be a global problem, because current nucleos(t)ide analogue therapy functionally reduces HBsAg level and suppresses HBV DNA but fails to control remission^[3,4]. Since obesity has emerged as a new global burden, NAFLD, which is one of the most common metabolic syndrome manifesting in liver with estimated prevalence of 20%~30% in many countries, will rank as the leading cause of HCC in future decades^[5-9].

Although patients diagnosed with early stage of HCC undergo surgical resection or radiofrequency ablation for curative intent, up to 70% of them are susceptible to recurrence within 5 years^[10]. During the last decades, conventional chemotherapy and combination of transarterial chemoembolization and sorafenib have been developed for patients at intermediate and advanced stages, however, therapy efficacy and adverse effects should be further assessed. Although sorafenib has been approved for treatment of HCC, the tyrosine kinase inhibitors-induced adverse events and drug resistance still remain an issue during clinical management^[11]. Therefore, more novel target agents and immunotherapies are being investigated and incorporated to achieve better outcomes^[12,13]. Here, this review mainly discusses the current progress in molecular and cellular mechanisms of HBV- and NAFLD-related HCC respectively, specifically the immune responses in liver, along with current immunotherapy options.

DIRECT PRO-ONCOGENIC MECHANISMS

HBV

Genomic features are significantly different in the group of HBV-HCC compared with non-infected patients. These results suggest that HBV-related HCC use alternative mechanisms for tumorigenesis to some extent. A high frequency of p53 inactivation and stem cell genes overexpression provide a potential pathogenic link between impaired cell reprogramming and HBV infection. Notably, these observations have clinical implication since TP53 mutations were associated with poor prognosis only in HBV-related tumors.

Virally oncogenic proteins

HBV genome contains four overlapping open reading frames, including preS1/preS2/S, preCore/Core, X and Pol, encoding viral proteins. Among them, hepatitis B X protein (HBx) is the most important oncogenic protein. Mutated or deleted HBx is frequently detected in HCC and plays critical role in liver carcinogenesis^[14-16]. Although it is still debated, a growing body of evidence suggests that both wild type HBx and truncated HBx promote tumorigenesis by abrogating cell-cycle arrest and apoptosis inhibition^[17-19]. HBx promotes HCC progression mainly through interaction with host factors including proteins and non-coding RNAs. HBx mutants interact with Bcl-2 and farnesoid X receptor leading to enhanced carcinogenesis^[20-22]. HBx-cortactin (CTTN) interaction promotes HCC progression by up-regulating expression of CREB1 and its downstream targets, cyclin D1 and MMP9^[23]. As a major conserved cellular pathway that controls critical cell processes, the ubiquitin proteasome system is often hijacked by HBx, leading to dysregulated ubiquitination. HBx stabilizes critical transcriptional oncoproteins Myc and PAX8 via blocking Skp2-mediated ubiquitination^[24,25]. Silencing lncRNA-MALAT1/miR-124 axis or miR-5188-FOXO1/ β -catenin-c-Jun feedback loop significantly block HBx-mediated upregulating stemness markers and reprogramming

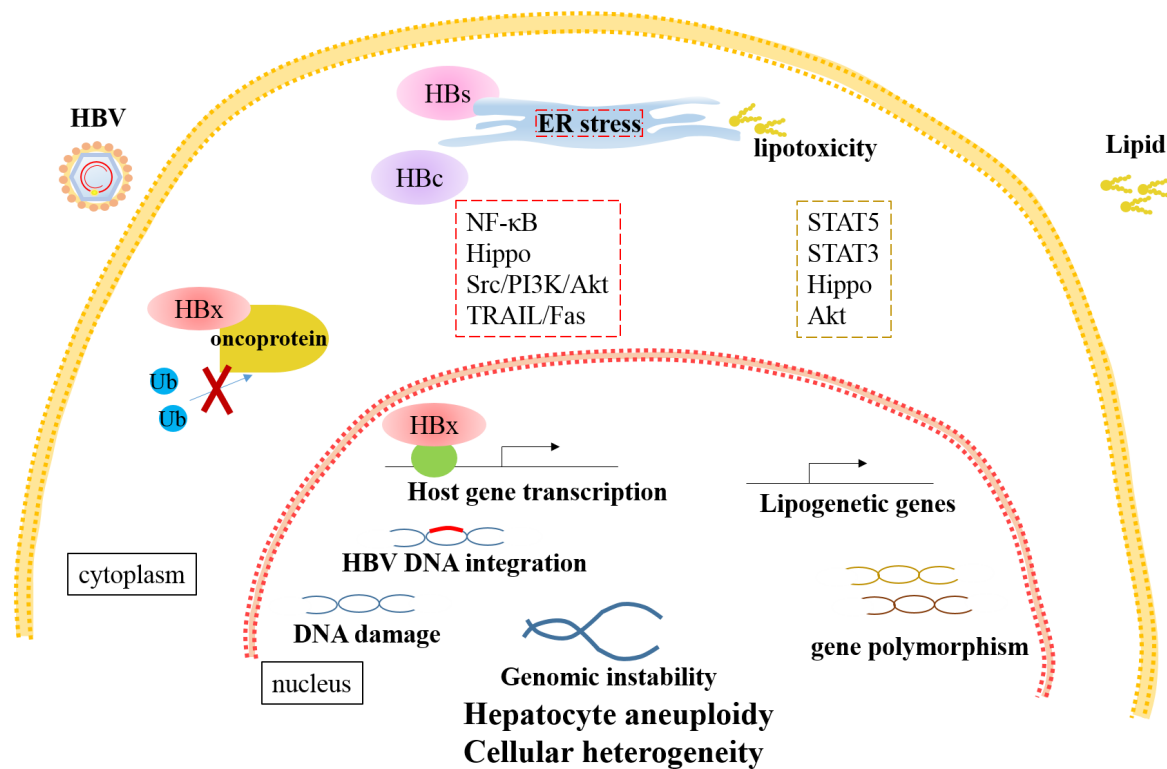


Figure 1. Direct oncogenic mechanisms of HBV- and NAFLD- HCC. HBV-encoded proteins lead to cellular heterogeneity via multiple manners. HBx stabilizes oncoproteins by suppressing ubiquitination or regulates transcription of host genes through transactivation. Cytoplasmic accumulation of HBs induces ER stress and thereby initiates cellular hippo pathways. Besides ER response, NF- κ B, Src/PI3K/Akt and TRAIL/Fas pathways are involved in HBc-induced abnormal proliferation and metabolism of hepatocytes. Furthermore, HBV DNA integration and viral protein result in DNA damage and genomic instability, which drive cellular heterogeneity. Excessive lipid accumulation stimulates ER stress and dysregulates apoptosis and fibrogenesis via STAT3 and hippo pathways. The STAT5 and Akt pathways are involved in upregulation of the lipogenic genes and downstream targets. Moreover, gene polymorphism is significantly related with NAFLD progression. HBV: hepatitis B virus; NAFLD: nonalcoholic fatty liver disease; HCC: hepatocellular carcinoma; ER: endoplasmic reticulum

proteins in HCC^[26,27]. Besides, HBx targets genes at transcriptional level. HBx complexed with HDAC1 binds to glycogen synthase 2 (GYS2) and transcriptionally inhibit the expression^[28] [Figure 1].

As the most abundant proteins, HBV surface (HBs) proteins are composed of three proteins, the large (L), middle (M), and small (S) HBs, encoded by the preS1/preS2/S gene respectively^[29-31]. Particular deletion mutants in preS2 have been reported to be associated with progression of chronic liver diseases. Mutated HBs accumulated in hepatocytes induce endoplasmic reticulum (ER) stress and initiate multiple cellular signal pathways, leading to cell growth advantages^[32,33]. Intrahepatic LHBs induce DNA damage and up-regulation of Plk1 to provoke hyperploidy, which disrupts the genomic stability of host cells^[34]. Pre-S2 deletion mutant also promote carcinogenesis by up-regulating expression of various oncogenes, including hTERT, TAZ in hippo pathways, and GPC3^[35-38] [Figure 1].

As the major capsid protein and important viral replication mediator, HB core-related antigen (HBcAg) is reported to be an independent risk factor for HCC development^[39]. Mechanistically, hepatitis B core protein (HBc) function as an immunogen as well as an important mediator of hepatocarcinogenesis via several mechanisms. HBc mutations P5T/H/L stimulate ER response, further increasing production of radical oxidative species (ROS) and activating NF- κ B signaling pathway, which directly promote infected hepatocytes to malignant transformation. Furthermore, HBc promotes proliferation, glycolysis and amino acid metabolism or suppress apoptosis via regulating Src/PI3K/Akt pathway, blocking TRAIL/Fas pathway or expression of p53 in transcriptional/post-transcriptional manner^[40-44] [Figure 1].

HBV DNA integration

HBV DNA integration occurs early once HBV entering into hepatocytes and remains stable throughout tumor progression, which explains the existence of monoclonal or polyclonal origins of HCC from the view of virus infection^[45,46]. HBV DNA susceptiblely integrates into rare fragile sites or functional genomic regions, which are proximate to protein coding or non-coding genes. Functionally, the protein-coding genes are deeply associated with tumorigenesis and the non-coding genes are involved in telomere maintenance, protein modification processes, and chromosome localization^[47-49]. For example, integration drives mutation in tumor suppressor gene *ZNF717* and over-expression of critical oncogene *c-MYC*. Meanwhile, integration into intron of cyclin A2 generates a pseudo-exon forming a chimeric fusion with *CCNA2*, which encodes protein promoting cell cycle progression^[46,50,51]. Moreover, HBV DNA integration is closely correlated with gender-bias and recurrence of HCC. Androgen receptor, but not estrogen receptor, enhances transcription responsive of TERT promoter with HBV integration to sex hormones via hepatocyte nuclear factor 4 alpha (*HNF4α*)- dependent manner^[52]. Multiple studies have identified fragile integration sites more in non-cancerous tissue than those in neoplastic tissues, which implies tumor recurrence^[48,53].

Genomic instability

In infected hepatocytes, both HBV DNA integration and viral proteins lead to genomic instability. Sequencing analysis of 373 liver cancer samples demonstrated ultra-high structural instability and preserved un-methylation in HBV integrated regions^[54]. HBx directly induces genomic instability via inhibiting mono-ubiquitylation of an evolutionarily conserved E3 ligase and impairing homologous recombination, which contribute to tumorigenesis^[55]. LHBs are the major viral protein leading to genomic instability. By inducing DNA damage and G2/M checkpoint failure, LHBs promote formation of hepatocyte aneuploidy and further self-propagating cycles of chromosomal instability, which drives cellular heterogeneity and clonal cancer evolution^[56,57].

NAFLD

NAFLD and its complication nonalcoholic steatohepatitis (NASH) are becoming the leading cause of HCC. Multiple pathways, such as abnormal metabolism, dysbiosis of gut microbiota and dysregulated immune responses, are involved in NAFLD initiated hepatocarcinogenesis and have been well summarized recently^[58]. Here, we mainly focus on dysregulated pathway mediated by lipid accumulation which is fundamental in progression of NAFLD related HCC. The abnormal intrahepatic lipid metabolism invokes insulin resistance, alteration of signaling pathways and oncogenes, followed by inflammation, fibrogenesis and hepatocarcinogenesis. In clinical samples, level of p-STAT5 is positively correlated with expression of sterol regulatory element binding protein-1 (*SREBP1*) and further *in vivo* and *in vitro* essays demonstrate that mTORC1 interacts and phosphorylates STAT5 to upregulate expression of lipogenetic genes, including *SREBP1*, fatty acid synthase (*FASN*) and acetyl-CoA carboxylase (*ACC*)^[59]. Both oxidative and ER stress caused by chronic lipotoxicity play critical roles in NAFLD-HCC. Oxidized LDL (oxLDL) uptake triggers CEBPβ expression to directly upregulate Nogo-B, an ER-residential protein, and promote lipophagy leading to lysophosphatidic acid-enhanced YAP oncogenic activity^[60]. In addition to hippo signaling pathway, STAT3-mediated pathways are frequently involving in pathogenesis of NAFLD-HCC, which mediated lipid accumulation, apoptosis and fibrogenesis^[61-66].

Other than metabolic disorder, gene polymorphism is significantly responsible for NAFLD. Recent genome-wide association studies (GWASs) in European ancestry suggest a robust relationship of *PNPLA3* gene cluster with NAFLD activity, progression to HCC and liver-associated death^[67-69]. This study also demonstrated an association of novel loci near *IL17RA* and *ZFP90-CDH1* with NAFLD disease severity and fibrosis^[67]. rs368234815 variant in *IFNL4* and *FNDC5* rs3480 polymorphism are respectively identified to be associated with liver damage and fibrosis in patients with NAFLD^[70,71]. In addition, gender-bias is as well observed in NAFLD-HCC. As the confirmed risk factor for male HCC, androgen receptor (*AR*) transcription activity is enhanced by key enzymes and specific unsaturated fatty acid produced in lipogenesis via activation of Akt kinase^[72] [Figure 1].

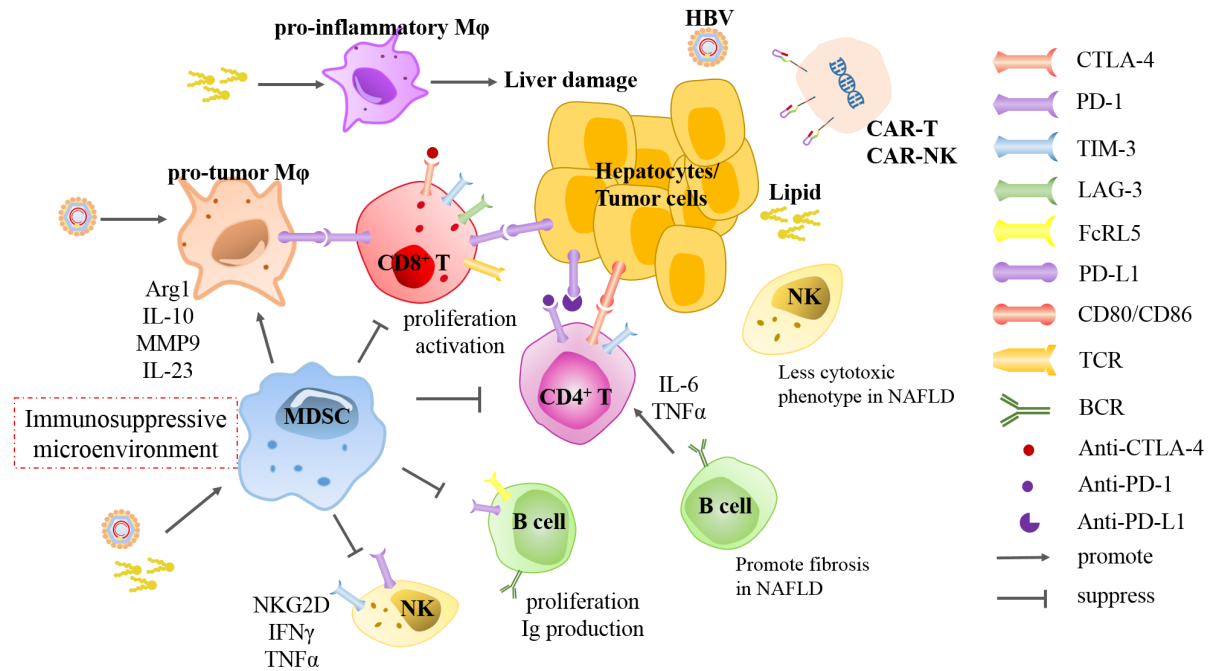


Figure 2. Alteration of immune cells in HBV- and NAFLD- HCC and corresponding immunotherapies. In HBV-infected liver microenvironment, MDSCs are recruited and macrophages are educated to polarization of pro-tumor phenotype, which constitute immunosuppressive microenvironment. However, excessive lipid accumulation and lipotoxic hepatocytes induce macrophages to pro-inflammatory phenotype which promotes liver damage. In both etiologies- associated HCC, cytotoxic T cells and NK cells inhibit progression of tumor formation through cytokines secretion or phenotype switch. B cells control progression of HBV infection by antibodies production and cytokines secretion, whereas promote NAFLD development towards fibrosis via inducing CD4⁺ T cells. Overall, activation and function of effector immune cells are suppressed by MDSCs and macrophages through interaction of checkpoint molecules, such as PD-1, PD-L1, CTLA-4, TIM-3 and LAG-3. Furthermore, several drugs blocking inhibitory checkpoint receptors have been developed and approved for treatment of cancer diseases. Collaboratively, CAR-T and CAR-NK cell therapy are investigated to be promising in treatment of liver cancer. HBV: hepatitis B virus; NAFLD: nonalcoholic fatty liver disease; HCC: hepatocellular carcinoma; MDSCs: myeloid-derived suppressor cells; CAR: chimeric antigen receptor; NK: natural killer; Mφ: macrophage; Arg1: arginase 1; MMP9: matrix metalloproteinase 9; IL: interleukin; IFN: interferon; TNF-α: tumor necrosis factor-alpha; TCR: T-cell receptor; BCR: B-cell receptor

ALTERATION OF IMMUNE MICROENVIRONMENT

Multiple resident or migratory immune cells, including innate and adaptive immune cells, are responsible for maintaining immune homeostasis in the liver. In addition, the dysregulation of hepatic immune cells play critical roles in liver cancer pathogenesis. Liver is rich of innate immune cells including myeloid cells and innate immune cells. The myeloid cells contain macrophages, dendritic cells and myeloid-derived suppressor cells (MDSCs). Liver innate lymphocytes are represented by natural killer (NK) cells, NKT cells, mucosal associated invariant T cells and $\gamma\delta$ T cells. CD4⁺ T, CD8⁺ T and B cells comprise major adaptive cells in liver^[73]. Both virus infection and metabolic disorder invoke the immune environment from tolerogenic status to active inflammation, leading to further cellular injury, fibrosis and eventually hepatocarcinogenesis. Moreover, various subtype immune cells and related markers in tumor microenvironment are significantly predictors for clinical outcome of patients^[74]. Here, this part will discuss the similarities and differences of immune cells responses between HBV and NAFLD-associated HCC [Figure 2].

Macrophages

Macrophages comprise almost 20% of overall immune cells in liver, containing residential Kupffer cells (KCs) and migratory monocyte-derived macrophages. Hepatic macrophages are extremely plastic and accommodative to signals from the microenvironment. Both inflammatory stimuli and viral proteins reprogram macrophages towards M2-like tumor macrophages, which in turn promote HCC progression^[75-77]. In chronic HBV-infected tissue, macrophages are manipulated by HBV for persistent maintenance in liver.

HBV suppresses pattern recognition receptors (PRRs) sensing or downstream interferon (IFN) response in hepatocytes but activate macrophages to secrete cytokines at high titer^[78]. Furthermore, HBc protein is detected within macrophages in liver tissues from HBV-infected patients. Macrophages exposed to HBV or HBV-producing cells are prone to secrete more IL-10, which subsequently impairs lymphocyte activation, but less pro-inflammatory cytokines such as IL-6 and IL-1 β with property of inhibition on HBV infection^[76]. Accordingly, HBV drives macrophages to be suppressive immune cells. In offspring with horizontal transmission of HBV, HBeAg induces up-regulation of checkpoint molecular PD-L1 on macrophage and polarization to M2 protumor subtype, which impairs responses of CD8⁺ T cell to HBV and lead to virus persistence^[79]. Moreover, macrophages produce matrix metalloproteinase 9 (MMP9) and IL-23 under the stimulation of HBV, which blocks binding of IFN- α to IFNAR1 and facilitate tumor angiogenesis and progression^[80,81].

Contrarily, pro-inflammatory macrophages are essential for progression of NASH-associated HCC. In a high-fat diet (HFD) zebrafish model, hepatic macrophage morphology is changed and number of TNF- α positive positive macrophages is increased, and liver size is subsequently reduced in macrophage-ablation NAFLD/NASH associated HCC larvae but not in HCC or NAFLD alone^[82]. The process from NAFLD to HCC is proximately accompanied by inflammation and fibrosis, in which, innate and adaptive immune cells are enriched and trafficking in liver. A recent study demonstrates that KCs induce recruitment of platelet in early stage of NAFLD, which is associated with hepatic injury. Furthermore, antiplatelet treatment abrogates infiltration of CD8⁺ T cells, Ly6G⁺ granulocytes, especially CD11b⁺ F4/80⁺ monocyte-derived macrophages and KCs, which alleviates the progression of carcinogenesis^[83]. Mechanistically, excessive toxic lipids and cholesterol crystals accumulate in macrophages, forcing cells polarization to pro-inflammatory phenotype, and depletion of KCs dampens release of pro-inflammatory cytokines and liver damage^[84-87]. Meanwhile, lipotoxic hepatocytes activate macrophages polarization via releasing exosomes containing miR-192-5p^[88]. Generally, NF- κ B and JNK signaling pathways mainly mediate the activation of macrophages towards the pro-inflammatory phenotype. Stimulator of interferon genes (STING) invokes the polarization of macrophage to produce TNF- α and IL-1 β via JNK/NF- κ B pathways, which further modulate increase lipid deposition in hepatocytes and exacerbate progression of NAFLD^[89].

MDSCs

MDSCs, generated as immature myeloid cells from bone marrow, are the major immunosuppressive cells which inhibit T cells proliferation and function and enhance induction of Treg cells and tumor-associated macrophages in chronic inflammation and cancers. Multiple studies have reported that MDSCs are closely related with HBV persistence and HCC. In patients with chronic HBV infection (CHB), HBeAg induces expansion of monocytic MDSCs (mMDSCs) via indoleamine-2,3-dioxygenase (IDO), to suppress autologous T cell proliferation and IFN- γ production, favoring the establishment of tolerant immune environment^[90]. Similarly, HBsAg promotes differentiation of mMDSCs through activation of ERK/IL-6/STAT3 signaling feedback^[91]. Mechanistically, cell cycle-related kinase (CCRK), as a direct AR-regulated oncogene, mediates virus-host signaling to promote progression of HBV-associated HCC^[92], and further induces polymorphonuclear (PMN) MDSCs in HCC^[93]. Moreover, AR-CCRK pathway is consistently shared by tumorigenesis in NASH-related HCC. Induction of CCRK activates mTORC1/4E-BP1/S6K/SREBP1 cascades and recruits PMN MDSCs to initiate metabolic reprogramming and immunosuppressive microenvironment to facilitate the progression of HCC^[94]. Concordantly, hepatic lipid deposit promotes accumulation of MDSCs and further enhances the cellular production of ROS in NASH-model mice^[95,96].

NK cells

Lymphocytes represent another crucial population in intrahepatic innate immune cells, including NKT cells, ILCs (NK cells and other ILC subpopulations) and $\gamma\delta$ T cells. NKT and NK cells, which mainly contain conventional circulating NK and liver-resident NK cells, comprise about 50% of lymphocytes in liver. Generally, hepatic NK cells recognize and restrain virus-infected cells via direct cytotoxicity or secreting

immunoregulatory cytokines, such as IFN- γ and TNF- α , to activate T cells^[97,98]. Recent studies demonstrate that liver-resident CD56 (bright) NK cells, which show increased expression of NKG2D and TRAIL and low production of IFN- γ , represent a cell population adapted for tolerogenic liver microenvironment and inducible anti-viral immunity^[99,100]. Accumulating evidence illustrates that function of NK cells is impaired in CHB and HBV progressed diseases. NK cells in blood from CHB patients express higher level of suppressive cell surface molecules and cytokines, such as Tim-3, PD-1 and IL-10, which is induced by HBsAg-mediated increase of monocytes^[101,102]. Besides, HBV nucleic acids-contained exosomes entering into NK cells inhibit expression of pattern-recognition receptors and thus NF- κ B and p38 MAPK pathways, which results in dysfunction of NK cells^[103]. Furthermore, intrahepatic NK cells-mediated apoptosis of hepatic stellate cells (HSCs) is reversed via blockade of TGF- β in HBV-infected liver cirrhosis patients, which leads to impairment of anti-fibrosis capacity in NK cells^[104]. In HBV-associated HCC, LINC01149 variant upregulates MICA expression through serving as miR-128-3p sponge to recruit NK cells to lyse infected cells, which process releases highly soluble MICA and thus induces exhaustion of NK cells^[105].

IL-15 is crucial for homeostasis of NK cells. Deficiency of IL-15 or IL-15R α inhibits high fat diet-induced accumulation of lipids and inflammation in liver^[106], which probably suggests dysregulation of NK cells involving in NAFLD progression. Although NK cells is a cell population with cytotoxicity in infected tissues, these cells convert to less cytotoxic ILC1-like phenotype in NAFLD, which probably protect obese liver from severe NAFLD but dampened capability to kill cancer cells^[107]. In advanced stages of fibrosis in NAFLD accompanied by insulin resistance, the ability of NK cells to restrain HSCs is impaired, mediated by low expression of insulin receptors, which leads to deterioration of the liver and fibrosis^[108]. Furthermore, NKp46⁺ NK cells, as immunoregulatory cells, induce polarization of hepatic macrophages towards M1-like phenotype via IFN- γ , which prevents NASH progression to fibrosis^[109].

T cells

T cells represent the major adaptive lymphocytes in transformation from inflammation to cancer. CD8⁺ T cells frequently show restricted proliferation and exhausted function with high expression of inhibitory checkpoint molecules such as CTLA-4, PD-1 and TIM-3 in CHB and HBV-HCC. High expression of PD-1 on HBV-specific T cells or B cells induces exhaustion of T cells or reduced antibodies production, which is partially reversed by PD-1 blockade^[110-112]. In HCC tissue, there exist CD8⁺ T cells expressing different levels of PD-1. The population with high-level PD-1 expresses TIM-3 and/or LAG-3 and produce limited IFN γ and TNF α when exposed to anti-CD3^[113]. Beyond, thymocyte selection associated high mobility group box (TOX) in CD8⁺ T cells promotes PD-1 translocation to cell surface by regulating endocytic recycling of PD-1^[114].

Abundant exhausted CD8⁺ T cells and Tregs exist and potentially clonally expand in cancerous tissues from HCC patients^[115], contributing to evasion of tumor cells from immune surveillance. However, several studies illustrate that CD8⁺ T cells potentiate to promote persistent hepatic inflammation susceptible to HCC development when HBV-specific CD8⁺ T cells stay in activation but lack of capability to constrain virus replication^[116]. Consistently, CD8⁺ T cells possess dual functions in progression of NAFLD-related HCC. In diet-induced obese mice, CD8⁺ T cells are recruited to liver to promote insulin resistance and glucose metabolism, leading to steatohepatitis^[117]. Meanwhile, another study demonstrates that activation of CD8⁺ T cells is suppressed by liver-resident immunoglobulin-A-producing (IgA⁺) cells, which express high levels of PD-L1 and IL-10^[118].

Compared to CD8⁺ T cells, HBV-specific CD4⁺ cytotoxic cells in PBMCs from HBV-associated HCC present at the similar level, but display weaker cytolysis capability and suppress cytotoxicity of CD8⁺ T cells in the absence of Tregs^[119]. The antigen-experienced T cells, CD4⁺ follicular helper T (Tfh) cells diminish HBV via response to HBsAg, which is impaired by CTLA-4-mediated Treg suppression^[120]. Contrary with CTLA-4, increased expression of co-stimulatory molecule OX40 on peripheral CD4⁺ T cells is associated with HBV

clearance^[121]. Furthermore, combined OX40 stimulation and PD-L1 blockade effectively activate IFN- γ producing Th1 cells and IL-21 producing Tfh cells, which potentiates the application of immunotherapeutic approaches^[122]. Consistent with anti-tumor function in HBV-related HCC, surveillance from CD4⁺ T cells are crucial for NAFLD^[123,124]. However, selective loss of CD4⁺ T cells has been recently detected in NAFLD, which promotes hepatocarcinogenesis^[125]. Mechanistically, linoleic acid (C18:2) is released from dead hepatocytes caused by lipotoxicity into CD4⁺ T cells. C18:2 then co-localizes within mitochondria, upregulating expression of carnitine palmitoyltransferase, which further promotes production of ROS and cell apoptosis^[124].

B cells

HBV viral load as well as HBsAg and HBeAg levels differed with progression of distinct phases of chronic HBV infection, accompanied by alteration of immune cells. Immunoglobulin-encoding genes and B cell function-related genes are more enriched in immune active patients than those in immune tolerant and inactive carrier patients^[126]. B-cell response and humoral immunity are essential for controlling progression of chronic HBV infection^[127,128]. However, differentiated HBsAg-specific B cells from patients with CHB are defective in antibody production and express high levels of inhibitory receptors, such as PD-1, FcRL4 and FcRL5^[112,129]. Blockade of PD-1 on B cells effectively reverses cellular dysfunction to maturation and cytokines secretion^[111]. Mechanistically, HBsAg inhibit toll-like receptor (TLR) promoter activity by suppressing phosphorylation of CAMP responsive element binding protein (CREB), thereby comprehensively inhibits expression of TLR9 in B cells, leading to reduced proliferation of B cells and decreased proinflammatory cytokines secretion^[130].

Intrahepatic B cells are highly associated with NAFLD. CD20⁺ B cells are increased in liver tissues from NAFLD patients with activity score higher than 5, compared with those of 0 or 1~4^[131]. Consistently, hepatic B cells account for more in HFD-induced NAFLD mice than in control mice. Moreover, the B cells in NAFLD liver tissues potentiate to produce higher levels of IL-6 and TNF- α , and promote differentiation of CD4⁺ T cells to Th1 cells^[132]. Functionally, the maturation of hepatic B2-cells and the increase of IgG in circulation targeting oxidative stress-derived epitopes predict the onset of steatohepatitis, and B cells-mediated activation of Th1 cells contributes to NAFLD progression toward fibrosis^[133].

IMMUNOTHERAPIES

As discussed above, a long duration is taken for both HBV- and NAFLD-related HCC, therefore, treatments aiming at eliminating HBV infection or blocking NASH progression and fibrogenesis can prevent the development of tumors. Abundant alterations of hepatic immune cells during liver carcinogenesis provide wide-range targets. Several immunotherapies have been approved for clinical application, nonetheless, the induced resistance and adverse events remain to be further investigated. The following sections will discuss immunotherapies in HCC, such as immune checkpoint inhibitors, virus or tumor vaccines, and adoptive cells transfer.

Immune checkpoint blockade

Inhibitory checkpoint receptors (ICRs) including CTLA-4, PD-1 and PD-L1, are recognized as master regulators for anti-tumor immunity. Immune checkpoint inhibition (ICI) therapy using antibody against PD-1, PD-L1 and CTLA-4 have been since been approved by the Food and Drug Administration^[134]. Moreover, function of exhausted CD8⁺ T cells is restored through implementation of anti-PD-1 therapy and even combined blockade of ICRs^[113,114]. In a cohort study with 262 patients enrolled between 2012 and 2016, nivolumab, a monoclonal antibody against PD-1, showed a decent safety profile, including acceptable tolerability^[135]. Consistently, safety and efficacy of nivolumab was comparable in Asian patients, compared to intent-to-treat overall population^[136]. However, a phase III trial showed that pembrolizumab, another PD-1 inhibitor, did not display significant increase in overall survival (OS) and progression-free survival

(PFS), compared with control groups^[137]. Since medicines based on anti-PD-L1 monoclonal antibodies have been validated to be effective for several cancer diseases, a phase Ib clinical trial of atezolizumab combined with bevacizumab, the anti-PD-L1 antibody and VEGF blocking antibody respectively (ClinicalTrials.gov NCT02715531), has announced an overall response rate of 32% among 73 patients with advanced HCC^[138]. Moreover, this combined therapy-based IMbrave150 phase III trial (ClinicalTrials.gov NCT03434379) is currently implemented and significant improvements in OS and PFS compared with sorafenib have been reported by the sponsor^[139,140] [Figure 2].

CTLA-4 is another inhibitory molecule that attenuates activation and function of T cells. Upregulation of CTLA-4 promote the apoptosis, secretion of anti-inflammatory cytokines and exhaustion of CD4⁺ Th and CD8⁺ T cells in liver tissues from CHB and HCC patients^[141-144]. Tremelimumab, which is the monoclonal antibody blocking CTLA-4, was administered in HCV-related HCC patients and assessed to possess antitumor and antiviral activity, along with good safety profile. The disease control rate was 76.4% and time to progression was 6.48 months (95%CI: 3.95-9.14)^[145]. The similar efficacy displayed in another cohort with 32 HCC patients enrolled. Combinational administration of Tremelimumab and tumor ablation results in accumulation of intratumoral CD8⁺ T cells and median OS of treated patients was 12.3 months (95%CI: 9.3 to 15.4 months), which is a potential new therapy for patients with advanced HCC^[146]. Furthermore, a preclinical study of durvalumab combined with tremelimumab was analyzed in patients with unresectable HCC (11 HBV⁺, 9 HCV⁺, 20 uninfected). Among 40 patients, 60% (24/40) had discontinued treatment. The uninfected patients after treatment showed predominantly clinical activity and no unexpected safety signals were observed. The phase II portion of this study is still ongoing^[147] [Figure 2].

Adoptive cells transfer

Chimeric antigen receptor (CAR) therapy is a promising strategy for cancer immunotherapy. Accumulating evidence has demonstrated CAR-T cell therapy as effective strategy against lymphoid leukemia and multiple myeloma^[148-150], whereas, efficacy and safety of CAR-T therapy for treatment of solid tumors remain elusive. Glypican-3 (GPC3) is broadly expressed on liver cancer cells, but minority on normal hepatocytes. GPC3-targeted CAR-T cells efficiently kill GPC3-positive hepatoma cells and the T cells expressing third-generation CAR potentiate to suppress growth of xenografts with even low GPC3 expression level in mice^[151]. Moreover, GPC3-specific CAR-T therapy combination with sorafenib could effectively promote apoptosis of liver cancer cells^[152] [Figure 2].

However, side effects to CAR-T therapy contain high manufacturing and fatal toxicities, such as cytokine release syndrome^[153]. CAR-NK therapy is under investigation and probably provide better options. Similar to CAR-T, FLT3- and CS1-targeted CAR-NK cells respectively suppress proliferation of cancer cells in myeloid leukemia and multiple myeloma^[154,155]. Furthermore, DAP12-targeted CAR-NK cells efficiently suppressed tumor cells in ascites and proliferation of liver-metastatic tumors in colorectal cancer patients^[156]. *In vivo* studies have shown that injection of ErbB2-specific NK-92/5.28.z cells possess the ability to prolong survival of glioblastoma-xenograft mice^[157] [Figure 2].

CONCLUSION

Chronic HBV infection-induced progression of HCC mainly depends on direct intrusion of viral components in hepatocytes, which disturbs its normal cellular metabolism and function. This subsequently stimulates immune imbalance in liver. Frequently accompanied by systemic metabolic syndrome, advancement of NALFD- associated HCC encompasses excessive lipid accumulation in hepatocytes and immune cells, insulin resistance and inflammation induced malignant transformation. The hepatic complexity of immune cells mediates tumorigenesis in situation of anergy of cytotoxic cells and enhancement of immunosuppressive cells. Cytotoxic T cells are the major factor which eradicates HBV-infected cells or malignant cells. The exhaustion mediated by MDSCs or Tregs leads to persistence of HBV and evasion of

cancer cells. Flexibility of macrophages facilitates themselves to polarize according to different signals from microenvironment. Macrophages mostly function as suppressive cells to inhibit proliferation and activation of CTLs in HBV-related HCC, while promotes liver damage and fibrosis via releasing pro-inflammatory cytokines.

Since there still remains lack of effective drug specifically targeting eradication of HBV or controlling development of NAFLD, illustrating molecular mechanisms of immune cells and establishing novel immunotherapies will provide promising options for treatment of HCC. Unbridling cytotoxic cells (ICI) and stimulating the cells specifically engineered with tumor-associated antigens (CAR-T and CAR-NK) mutually contribute to effectively restrain progression of HBV- and NAFLD-induced HCC. Taken together, the drug resistance and adverse effect induced by immunotherapies should be further recorded and investigated in future studies.

DECLARATIONS

Authors' contributions

Conceived of the presented idea: Ma CH, Song XJ

Performed the basic writing: Song XJ

Developed the further revision: Ma CH

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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