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Review

Natural killer cell dysfunction in hepatocellular carcinoma and NK cell-based immunotherapy

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The mechanisms linking hepatitis B virus (HBV) and hepatitis C virus (HCV) infection to hepatocellular carcinoma (HCC) remain largely unknown. Natural killer (NK) cells account for 25%–50% of the total number of liver lymphocytes, suggesting that NK cells play an important role in liver immunity. The number of NK cells in the blood and tumor tissues of HCC patients is positively correlated with their survival and prognosis. Furthermore, a group of NK cell-associated genes in HCC tissues is positively associated with the prolonged survival. These facts suggest that NK cells and HCC progression are strongly associated. In this review, we describe the abnormal NK cells and their functional impairment in patients with chronic HBV and HCV infection, which contribute to the progression of HCC. Then, we summarize the association of NK cells with HCC based on the abnormalities in the numbers and phenotypes of blood and liver NK cells in HCC patients. In particular, the exhaustion of NK cells that represents lower cytotoxicity and impaired cytokine production may serve as a predictor for the occurrence of HCC. Finally, we present the current achievements in NK cell immunotherapy conducted in mouse models of liver cancer and in clinical trials, highlighting how chemoimmunotherapy, NK cell transfer, gene therapy, cytokine therapy and mAb therapy improve NK cell function in HCC treatment. It is conceivable that NK cell-based anti-HCC therapeutic strategies alone or in combination with other therapies will be great promise for HCC treatment.

Keywords: natural killer cell; hepatocellular carcinoma; HBV; HCV; immunotherapy; gene therapy; cytokine therapy; mAb therapy

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Introduction

Hepatocellular carcinoma (HCC) is currently the fifth most common malignancy and the third leading cause of cancer mortality[1]. The highest HCC incidence rates are reported from developing countries, where hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are endemic. The main risk factors for HCC include HBV/HCV infection, alcoholism, nonalcoholic steatohepatitis and diabetes[2]. Chronic HBV infection accounts for approximately 50% of HCC cases worldwide[3, 4]. Despite the accumulated clinical and epidemiological evidence showing that the development of liver cancer is highly related to chronic HBV infection, the mechanisms linking HBV infection and HCC remain largely unresolved. Natural killer (NK) cells, which are innate lymphoid cells that have natural cytotoxicity and regulatory functions, form the first line of defense against viral infections and tumors. The proportion of innate immune lymphocytes [including NK, NK T-cells (NKT) and γδT cells] accounts for 50% of the total number of liver lymphocytes, of which NK cells account for a large part[5]. The percentage of NK cells among liver cells is at least five times as high as the percentages among spleen or peripheral blood, suggesting that NK cells may play an important role in the immune function of the liver. NK cells constitutively express a large number of immune recognition receptors (NKRs) that recognize ligands on hepatocytes, liver sinusoidal endothelial cells (LSEC), stellate cells and Kupffer cells to maintain the balance between the immune response and immune tolerance[6, 7]. The number of NK cells in the peripheral blood of patients with HCC is significantly positively correlated with survival rates and the prognosis of liver cancer[8, 9]. Despite these associations, the mechanisms linking NK cells and HCC remain unclear. Here, we describe the abnormal numbers, phenotypes and dysfunctions of NK cells in patients with chronic HBV or HCV infection and HCC. Then, we summarize the current achievements in HCC immunotherapy by improving NK cell functions.

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Abnormal frequency and phenotype of NK cells in chronic hepatitis B (CHB) and chronic hepatitis C (CHC) patients

Chronic HBV and HCV infections are the leading causes of liver disease progression, resulting in hepatic cirrhosis and HCC. The incidence of HCC in the West and Japan is clearly rising due to chronic HCV infection. In developing countries, HBV infection comprises 70% of the risk factors for HCC[4]. Decreased NK cell activity in HCC patients might be related to chronic hepatitis B and C virus infection. The chronic inflammation induced by persistent HBV or HCV infection impaired NK cell function, thereby contributing to the progression of HCC.

NK cells are usually abundant and play an important role in early viral clearance in acute HBV- or HCV-infected patients; however, the numbers of NK cells are lower in both peripheral blood and the liver in chronically infected HBV and HCV patients compared with healthy individuals. Moreover, the frequency of NK cells appears to decrease concomitantly with disease progression[10–12]. The total proportion of NK cells in PBMCs from HCV patients was remarkably decreased compared with healthy controls (8.6% *vs* 13.3%, respectively). The frequency of $CD56^{bright}$ cells was increased (10.0% *vs* 6.0%, respectively), while the frequency of $CD56^{dim}$ cells was reduced (90.0% *vs* 94.0%, respectively)[12]. Another study found that the frequencies of circulating NK cells were reduced and the phenotypes were altered in 22 HBV^+ and 35 HCV^+ patients compared with healthy controls[11]. The percentage of peripheral blood NK cells was approximately 30% lower in the 28 HCV patients compared with the HCV-negative subjects. The reduction was mainly derived from the $CD56^{dim}$ NK cells[10]. In HCV patients, the proportion of intrahepatic $CD56^+$ NK cells was dramatically lower compared with their proportion in the peripheral blood (5.1% *vs* 8.6%, respectively). Similar reduced ratios of NK subsets in the liver and blood demonstrated that the decreased proportion of peripheral NK cells in HCV patients was not caused by their accumulation in the liver[13].

Persistent HBV or HCV infection often leads to changes in the phenotype of NK cells. In HCV patients, the frequencies of the HLA class I-specific receptors $CD158^{a,\,h+}$ and $CD158^{b,\,j+}$ on NK cells in liver infiltrating lymphocytes were significantly reduced, whereas intrahepatic $NKG2A^+$ NK cells were more obviously decreased in HBV patients[12]. The phenotypic changes observed in chronic HCV patients are controversial. Earlier reports analyzed NK cell phenotypes from peripheral blood. In contrast, most later reports analyzed intrahepatic NK cells or compared intrahepatic NK cells with blood NK cells, thereby showing different phenotypic characteristics between intrahepatic and blood NK cells. Most data showed that the expression of activating receptors (*eg*, NKp30, NKp46, NKG2D, and NKG2C) was increased, while the expression of inhibitory receptors (such as NKG2A) was decreased on intrahepatic NK cells from persistently infected HCV patients[11–14]. However, one report showed a significant reduction in the proportion of activating NK cell receptors (*eg*, NKp46 and NKp30)-expressing NK cells accompanied by an increased proportion of NKG2A-expressing NK cells in chronic HCV patients compared with healthy and HBV-infected subjects[15]. The controversy concerning phenotypic features might derive from patients with different stages of disease (acute or chronic infection), viral loads, HCV genotypes, sampling sites (derived from blood or liver tissue), or populations. Some reports analyzed smaller numbers of subjects, and some reports failed to include appropriate control groups. Indeed, the evaluation of intrahepatic NK cells in healthy donors is limited by obvious ethical reasons. For HBV persistence, most reports showed reduced expression of activating receptors and increased expression of inhibitory receptors on hepatic or peripheral NK cells. For example, NKG2D/DAP10 and 2B4/SAP expression on NK cells was found to be decreased, while NKG2A expression was significantly increased in patients chronically infected with HBV[16, 17]. The expression of the co-inhibitory receptor Tim-3 was reported to be significantly increased on circulating NK cells and liver-infiltrating lymphocytes from 40 CHB patients compared with 18 healthy controls and nine patients with fatty liver disease[18]. Another co-inhibitory receptor (PD-1) was also found to be up-regulated on intrahepatic NK cells and other immune cells from patients chronically infected with HBV[19].

Functional impairment of NK cells in CHB and CHC patients

The phenotypic changes in NK cells induced by chronic HBV or HCV infection are usually accompanied by, or lead to, NK cell dysfunction[16, 20]. Most observations demonstrated that the cytotoxicity and production of IFN-γ and TNF-α by NK cells were reduced during chronic HCV infection. However, some results showed that phenotypic changes did not necessarily reflect altered functions. The functional dichotomy of NK cells has also been reported in chronic HBV and HCV infections. For example, the expression of activating receptors (particularly NKG2D) was increased and the expression of inhibitory receptors was decreased in HCV patients, while the expression of activating receptors (particularly NKG2C) was increased and normal expression of inhibitory receptors was observed in chronic HBV patients; nevertheless, peripheral NK cells displayed enhanced cytotoxicity, whereas the production of IFN-γ and TNF-α was reduced in both chronic HBV and HCV patients[11]. CD107a degranulation and TNF-related apoptosis inducing ligand (TRAIL) expression were increased during chronic HBV and HCV infection, along with suppressed production of IFN-γ and TNF-α[11, 14, 21]. These functional features of NK cells in combination with conserved or enhanced cytolytic activity and dysfunctional cytokine production might contribute to chronic liver immunopathology and virus persistence. Anti-viral therapy or blockade of immunosuppressive cytokines (IL-$10^{+/-}$ TGF-β) restored NK cell activation and IFN-γ production[21, 22]. However, another report showed significantly dysfunctional intrahepatic NK cell cytotoxicity associated with reduced expression of TRAIL and CD107a in chronic HCV patients compared with healthy controls[23]. Significant production of

IL-10 and normal concentrations of IFN-γ by freshly separated NK cells from HCV patients were also reported[24]. Based on comparisons of the characteristics of the patients studied, the discrepancies concerning NK cell functions in chronic HCV patients might be associated with differences in viral loads, HCV genotypes, control groups, or detection methods, similar to the controversial reports concerning NK cell phenotypes. Regardless, most reports demonstrated impaired function, and even functional exhaustion, of NK cells during persistent chronic HBV or HCV infection that contributed to the progression of HCC.

Abnormal proportion or frequency of NK cells in HCC

Abnormal NK cytolytic functions have been described in various mouse models of cancer and in human patients[25–28]. NK cell exhaustion is highly correlated with the progression and metastasis of a variety of tumors[29, 30]. Earlier observations about the role of NK cells in HCC progression were derived by detecting the proportion and absolute number of NK cells in peripheral blood. A reduction in the proportion of peripheral blood NK cells has been found in HCC patients at various stages compared with healthy controls. Specifically, peripheral $CD56^{dim}CD16^{pos}$ NK subsets displayed a dramatic reduction, which resulted in a dramatically increased ratio of $CD56^{bright}$ and $CD56^{dim}$ NK cells in HCC patients[31]. Reduced absolute counts of $CD56^{bright}$ and $CD56^{dim}$ NK cells were also observed in the peripheral blood from 20 Egyptian patients with hepatitis C-related HCC compared with 152 healthy control subjects[32]. Later investigations focused on liver-resident NK cells in HCC patients to reflect the physiological nature of NK cells in the tumor environment. The lack of healthy livers meant that most of these studies compared tumor infiltrating lymphocytes (TILs) with non-tumor infiltrating lymphocytes (NILs). Cai *et al* analyzed 110 HCC patients at various stages (I/II/III) and found that NK cells largely accumulated in the liver tissues of HCC patients whose total peripheral NK cell frequencies were significantly decreased, whereas more NK cells were detected in NILs (approximately 19.7%) than in TILs (approximately 5.34%). There was no difference in the proportion of $CD56^{bright}CD16^{neg}$ NK cells between NILs and TILs in these HCC patients. The dramatic reduction in the frequency of tumor-infiltrating NK cells mainly reflected the reduction in $CD56^{dim}CD16^{pos}$ NK cells[31]. Another study reported a similar result: a decreased intrahepatic NK cell frequency in TILs (20.6%±10.4%) compared to NILs (27.9%±13.5%) in 50 HCC patients in various stages of disease (I/II/III/IV: 15/23/8/4)[33]. The NK proportion in TILs tends to decrease in advanced stage HCC patients. Another interesting study investigated the infiltration of NK cells in normal human livers, chronic hepatitis livers, intratumoral livers and their paired nontumoral livers using immunohistochemical staining. The results showed that NK cells were predominant in the normal liver, chronic hepatitis liver and nontumoral liver compared with the intratumoral tissue. The reduction in NK cells was particularly noticeable in patients with advanced stage HCC[34]. Furthermore, the density of the infiltrating $CD56^+$ NK cells was correlated with HCC patient survival[9]. These data demonstrated a reduction in the frequency of circulating and intrahepatic NK cells in HCC patients that might contribute to the tumor's escape from immune surveillance.

Functional impairment of NK cells in HCC patients

Although there was a striking positive correlation between NK cell density in the intratumoral region and survival, NK cells often exhibited reduced infiltration and impaired functional activities (*ie,* lower production of TNF-α and IFN-γ) in advanced-stage HCC patients[31]. Increasing evidence strongly suggested that the dysfunction or exhaustion of NK cells in advanced tumor sites in HCC patients contributed to the pathogenesis of liver cancer. Several studies have shown that NK cells from PBMCs and TILs in HCC patients were defective in cytotoxicity and cytokine secretion compared with NK cells from healthy donors, and decreased NK cell activity might be associated with the development and invasion of HCC[8, 31, 35, 36]. The defect in NK cell activity was inversely correlated with the patient's age, and HCC patients with lower NK cell activity usually presented venous invasion or involvement of both lobes[36]. The cytotoxicity of NK cells from both the peripheral blood and tumor sites of HCC patients was notably reduced compared with healthy controls[8]. The production of cytoplasmic granules (*ie,* granzyme A, granzyme B and perforin) and the secretion of IFN-γ by NK cells from PBMCs were also substantially decreased in advanced HCC patients (stage II and III) compared with healthy donors. Similar decreased activity was also found in NK cells from TILs compared with NILs. Significant reductions in granzyme B and perforin were also noted when stage II patients were compared with stage I patients. The reduced NK cell activity was associated with increased $CD4^+CD25^+$ T regulatory cells in the tumor environment of HCC patients[31]. Myeloid-derived suppressor cells (MDSCs), monocytes/macrophages and fibroblasts derived from HCC also trigger NK cell dysfunction via the NKp30 receptor, CD48/2B4 or PGE_2 and IDO separately[8, 34, 37].

NK cell-based immunotherapy for HCC

The development and progression of tumors is dependent on not only the intrinsic genomic instability of the tumor but also is the result of co-evolution of the immune system and tumor cells. The critical role of NK cells in the defense against viral infections and tumors has been confirmed. Massive tumor growth has been observed in NKG2D-deficient mice, providing critical evidence for the surveillance of tumors by NK cells and their receptors[26]. The therapeutic strategy of targeting NK cells in human cancer immunotherapy has displayed an efficient effect, particularly in hematological malignancies. This approach also shows promise in solid cancers, such as metastatic melanoma, kidney cancer and lung cancer in addition to HCC. Due to the rich frequency and unique features of NK cells in the liver, they exert important effects on the development and progression of HCC, in particular by restricting HBV or HCV infection and preventing disease

progression from chronic hepatitis to HCC[38, 39]. NK cells accumulate within the liver and can directly (or indirectly through the production of cytokines) kill infected cells, stressed hepatocytes and tumor cells. NK cells can also kill activated hepatic stellate cells (HSCs), thereby alleviating liver fibrogenesis. Accordingly, NK cells play crucial roles in not only eradicating HBV or HCV from the infected hepatocytes but also suppressing the development of fibrosis, cirrhosis and HCC; however, their functions (*ie*, cellular cytotoxicity and cytokine production) are disrupted in patients with prolonged HBV/HCV infection and during tumor transformation. Therefore, NK cell-based immunotherapy to overcome the mechanism of NK cell dysfunction and to induce efficient NK cell immune responses has been exploited as a promising therapeutic strategy. The therapeutic strategies mainly focus on inducing NK cell activation and reversing the dysfunction of NK cells, and have shown exciting potential for HCC immunotherapy (Figure 1).

Chemoimmunotherapy

Currently, a number of useful therapies are available to patients diagnosed with liver cancer. The therapeutic options for advanced HCC include surgical resection, liver transplantation and percutaneous ablation. Sorafenib, a multikinase inhibitor, is the first and only molecularly targeted drug shown to have a survival benefit in patients and has become a standard treatment for advanced HCC[40]. In addition to its direct suppressive effect on the growth of hepatoma cells, sorafenib has recently been shown to trigger hepatic NK cell activation and induce NK cell antitumor responses by triggering the proinflammatory activity of tumor-associated macrophages (TAM)[41]. Sorafenib also enhanced NK cell-mediated cytotoxic activity against HCC by increasing the expression of membrane-bound MICA and decreasing levels of soluble MICA from HCC cells[42]. These studies suggested an important effect of NK cell activation on the therapeutic efficacy of sorafenib in HCC patients. Similarly, the proteasome inhibitor Bortezomib not only mediates an antitumor effect in HCC by inhibiting tumor cell proliferation but also stimulates the cytotoxicity and IFN-γ production of NK cells by augmenting the cell surface expression of MICA/B on hepatoma cells[43]. Histone deacetylase inhibitors (HDACi) are a new class of anticancer agents that have shown promise in clinical trials for the treatment of human malignancies, including HCC. In addition to their direct proliferation inhibition and apoptosis induction of tumor cells, increasing evidence has shown that HDACis promote the recognition of tumor cells by immune cells. We and others have reported that HDACis promote MICA or MICB expression on hepatoma cells and increase the susceptibility of hepatoma cells to NK cell-mediated lysis[44–46]. The HDACi suberoylanilide hydroxamic acid (SAHA) not only directly augmented MICA/B transcription via promotion of MICA/B-associated histone acetylation but also epigenetically repressed the transcription of MICA/B-targeting miRNAs (miR-20a, miR-93, and miR-106b) by downregulating the host miRNA genes (miR-17-92 cluster and MCM7)[46]. This novel mechanism of action further supports the promise for HDACis in the therapy of HCC by promoting the efficacy of NK cell-mediated immunotherapy. Taken together, the synergistic chemoimmunotherapeutic strategy that relies on priming NK cell-mediated antitumor immune responses has shown great promise for HCC treatment.

NK cell transfer

Currently, using autologously or allogeneicly *ex vivo* expanded NK cells for tumor therapy is becoming more attractive to

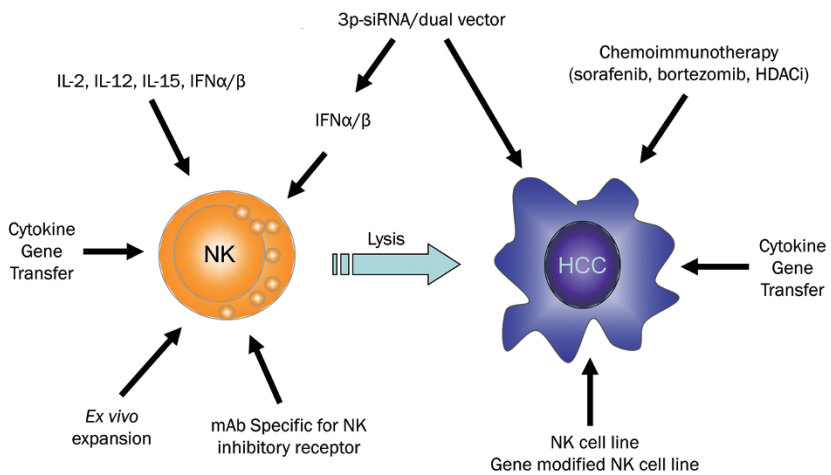

Figure 1. Natural killer (NK) cell-based immunotherapy for hepatocellular carcinoma (HCC). NK cell-based immunotherapy to overcome the mechanism of NK cell dysfunction and to induce efficient NK cell immune responses is a promising therapeutic strategy. The strategy involves chemoimmunotherapy, adoptive transfer of NK cells or gene-modified NK cells, gene therapy and cytokine therapy, and therapy with a mAb specific for NK inhibitory receptors. Combined therapy that boosts NK cell activation and blocks inhibitory signaling pathways (*eg*, PD-1, CTLA-4, and KIR) has been shown to be more effective at reversing tumor-induced immune tolerance. A recent dual functional therapy strategy comprising both the stimulation of NK cell activation by inducing type I IFN production and the targeted inhibition of tumor growth-related genes has shown great promise for HCC therapy.

researchers[47, 48]. NK cells from peripheral blood can be expanded by an average of 1600-fold using good manufacturing practices with compliant components[49–53]. Moreover, their cytotoxicity against primary autologous tumor targets is significantly increased with no significant cytotoxicity against normal cells. Cytokines such as IL-2, IL-12, IL-15, IL-21, and type I IFN further enhance the activation and cytolytic potency of NK cells during expansion[48]. The main limitation in using cytokines to stimulate endogenous NK cell activation or proliferation for cancer treatment is the toxicity of systemic cytokine administration. For example, adoptive transfer of autologous NK cells in combination with IL-2 often results in poor clinical outcomes due to the high toxicity of IL-2. Moreover, high IL-2 doses promote the expansion of regulatory T cells (Treg), thereby suppressing NK-cell functions, and inducing activation-induced cell death (AICD) of NK cells. IL-15 acts as an essential cytokine for NK cell development and survival that manifests antiapoptotic actions and inhibits IL-2-mediated AICD. Importantly, IL-15 shows potential for supporting large-scale expansion of clinical-grade NK cells with enhanced longer-term potency and preservation of activation receptors in therapy for malignancies without inducing the expansion of Tregs[49]. Another factor that impairs the efficacy of autologous NK cell transfer in cancer patients may be the MHC class I expression on tumor cells that suppresses the activation of autologous NK cells *in vivo*. Infusion of donor–recipient inhibitory KIR-HLA-mismatched allogeneic NK cells or blocking inhibitory receptors specific for MHC-I using anti-KIR Abs has shown strong clinical benefits with minimal toxicity in therapy for leukemia and solid cancers. A Phase I clinical trial on the safety and effectiveness of autologous NK and NKT cells to treat cancers such as HCC (registered at www.clinicaltrial.gov as trial NCT00909558) was sponsored in 2009, but was later suspended. A Phase II clinical trial (NCT02008929) to evaluate the safety and efficacy of MG4101 (*ex vivo* expanded allogeneic NK cells) as a secondary treatment after curative liver resection on patients with advanced HCC and a high risk of recurrence is about to begin. A safety study of liver NK cell therapy for hepatoma liver transplantation (NCT01147380) is also ongoing. Transfer of the NK92 cell line (the only NK cell line that has been approved by the FDA and has entered clinical trials) has shown efficient antitumor effects in patients with advanced malignant melanoma and renal cell carcinoma. NKG, a new NK cell line established in China, also exhibited strong cytotoxicity against human tumors *in vitro* and in a mouse xenograft model, showing promising adoptive immunotherapy for human cancer[54].

Cytokine gene modification of NK cells could promote NK cell proliferation, increase NK cell survival and enhance the anti-tumor activity of NK cells[55, 56]. Adoptive transfer of highly cytolytic NK cell lines and gene-modified NK cell lines has shown great promise in HCC therapy[57, 58] and the prevention of HCC recurrence[59]. In particular, a human IFN-α gene-modified NKL cell line (NKL-IFNα) showed strengthened cytolytic activity against human HCC cell lines and primary human hepatoma cancer cells compared with parental NKL cells. Importantly, adoptive transfer of NKL-IFNα cells significantly suppressed the tumor growth of HCC in a xenograft model and prolonged the survival of tumor-bearing nude mice[57]. Similarly, a human IL-15 gene-modified NKL cell line (NKL-IL-15) displayed a promising anti-HCC therapeutic effect in a xenograft model[58]. Adoptive transfer of TRAIL-expressing NK cells also improved the depressed immune status and prevented the recurrence of hepatocellular carcinoma after partial hepatectomy in a mouse model[59]. Similarly, adoptive transfer of granzyme H-overexpressing NK cells resulted in HBV eradication in HBV-infected mice[60]. Human allogeneic suicide gene-modified killer cells (aSGMKCs) were recently reported to show a high, rapid, interleukin-2-dependent, mainly NK cell-mediated cytotoxicity against human hepatoma cells, resulting in a marked, rapid and sustained regression of HCC *in vivo* after adoptive transfer[61].

Gene therapy and cytokine therapy

Some effective genetic manipulation approaches have been demonstrated to enhance the interaction of NK and tumor cells by expressing transgenic cytokines and overexpressing activating receptors. Intratumoral gene transfer of cytokine genes such as IL-2, IL-12, and IL-15 could induce NK cell proliferation, enhance their activation and further restore their cytotoxic capacity[62]. Gene therapy with an adenovirus carrying the IL-12 gene (AdCMVIL-12) for orthotopic HCC in rats demonstrated a significant inhibition of tumor growth. The key antitumor mechanism was the activation of NK cells by AdCMVIL-12[63]. Intratumoral gene transfer of IL-12 in a primary murine HCC model also significantly inhibited tumor growth, neovascularization and spontaneous lung metastasis. Similarly, the inhibition of tumor growth was almost entirely dependent on NK cells; this finding was confirmed by NK cell depletion[64]. The anti-tumor effect of adenovirus-mediated CD40L (AdCMVmCD40L) transfer in a rat HCC model was also associated with increased IL-12 serum levels and enhanced NK activity[65]. Adoptive transfer of IFN- or IL-15 gene-modified NK cells augmented anti-HCC effects in a xenograft model[57, 58]. An experimental therapy for HCC comprising type I and type III IFNs in a BNL hepatoma model demonstrated the critical role of NK cells in the anti-tumor activity of IFNs[66]. A recombinant vesicular stomatitis virus expressing IFN-β, which may exert an anti-tumor effect by activating NK cells, has been proposed; the Phase I clinical trial (NCT01628640) is now in progress to treat patients with adult primary HCC and sorafenib refractory/intolerant HCC. Another novel therapeutic strategy for HCC using a dual-function vector with both immunostimulatory and pim-3-silencing effects has shown great promise with *in vivo* anti-tumor effects by arousing NK cell activation via ssRNA-primed immunostimulation and type I IFN production[67]. This dual functional therapy strategy involving both stimulating immune responses (particularly NK cells) by inducing type I IFN production and targeting inhibition of tumor growth-related genes has shown great promise for treating both chronic HBV

infection and HCC[68–72].

mAb therapy

Therapeutic approaches targeting co-inhibitory molecules using specific monoclonal antibodies have opened up an exciting new era of immunotherapy[73–75]. The effectiveness of cancer immunotherapy targeting the co-inhibitory molecules CTLA-4 and PD-1 has been fully validated in advanced melanoma and other solid tumors, which demonstrated that the reversal of tumor immune tolerance is an important new pathway for the treatment of cancer. Human anti-CTLA-4 monoclonal antibodies were approved by the FDA in 2011[76]. The results from clinical trials of the use of anti-PD-L1 monoclonal antibodies to block the PD-1/PD-L1-mediated signaling pathway demonstrated that targeting co-inhibitory molecules represented an attractive immunotherapeutic approach[77, 78]. Furthermore, a combination of anti-CTLA-4 and anti-PD-L1 antibodies was more effective at reversing tumor immune tolerance in mice[79, 80]. Dual anti–LAG-3/anti–PD-1 antibody treatment resulted in decreased tumor growth and increased survival[81]. This therapeutic strategy has also begun clinical testing in advanced HCC patients and has shown promising effects. An anti-CTLA-4 human mAb (tremelimumab) has been used for advanced HCC and has completed its Phase II clinical trial (NCT01008358); this antibody displayed an acceptable safety profile as well as antitumor and antiviral activity[82]. A phase I trial of an anti-PD-1 mAb (nivolumab) is currently ongoing for patients with advanced HCC (NCT01658878)[83].

This immune checkpoint blockade usually targets T cells and aims to rescue T-cell exhaustion and amplify T cell responses. NK cells also express a variety of co-inhibitory receptors that deliver inhibitory signals. NK cell activation is regulated by the balance between inhibitory and activating receptors, with inhibitory signals typically predominating over activation signals by blocking activating signaling. Increased levels of inhibitory receptors and decreased expression of activating receptors on NK cells limit NK cell-mediated tumor immunosurveillance in HCC patients. The expression of the co-inhibitory receptors PD-1 and Tim-3 on NK cells in patients with HBV infection were significantly increased and were associated with the exhaustion of immune cells[84, 85]. Hence, these inhibitory receptors may also act as immune checkpoints in the regulation of NK cell activation and responses. Targeting co-inhibitory molecules on NK cells with specific monoclonal antibodies might represent a promising strategy for tumor therapy, in particular HCC. Therapeutic strategies that block inhibitory receptors (such as the killer immunoglobulin-like receptor, KIR) to enhance NK cell activity have been exploited in hematological diseases, such as leukemia, lymphoma and multiple myeloma (MM), and have shown efficacy and safety[86, 87]. This therapy, alone or in combination with anti-PD-1 antibodies, is also being tested in patients with advanced solid tumors (NCT01714739). Our recent report showed that blocking the inhibitory receptor NKG2A displayed efficacy for the promotion of NK cell activity and clearance of HBV in HBV-persistent mice[17]. The T-cell immunoglobulin and ITIM domain (TIGIT) receptor was recently defined as an inhibitory receptor expressed on NK cells[88]. We recently demonstrated the negative regulatory role of TIGIT on NK cell activation in a murine acute viral hepatitis and liver regeneration model[89, 90]. Blocking TIGIT enhanced NK cell activation and interferon-gamma (IFN-γ) production[89]. These results showed that TIGIT might be a candidate immune-checkpoint protein, and blockading TIGIT to reverse dysfunctional NK cells might be useful for HCC therapy. Collectively, an inhibitory blockade for NK cells might become a promising therapeutic strategy for HCC and chronic HBV or HCV persistent patients, although further study is required to confirm its potential value.

Conclusion and perspectives

NK cells play crucial roles in the clearance of HBV or HCV infection and suppression of the development of liver fibrosis, cirrhosis and hepatocarcinogenesis. NK-cell-based anti-HCC therapeutic strategies are becoming increasingly attractive. However, most attempts are being explored in animal models or in ongoing human clinical trials. Assessment of the long-term anti-tumor effects and safety require further observation. An enhanced understanding of tumor recognition by NK cells and the mechanisms used by HCC to evade NK cells within the liver-specific microenvironment will yield valuable insights for the treatment of HCC. The selection of NK cells with a predominant activating profile (*eg*, the selection of KIR-mismatched NK donors or the blockade of inhibitory checkpoint molecules on NK cells) is critical for delivering successful anti-HCC activity. Immunotherapy based on specific NK subsets (particularly liver-specific NK cells) may represent an attractive approach for HCC patients. Importantly, strategies to combine NK cell-based immunotherapy with conventional chemotherapy or other multiple therapies will hold greater promise for HCC and HBV/HCV persistent patients.

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