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Mini-review

Immunotherapy of hepatocellular carcinoma using chimeric antigen receptors and bispecific antibodies

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most lethal cancers worldwide with an overall survival rate of less than 15% in developed countries. Despite attempts at new therapeutic strategies, the majority of patients succumb to this cancer. Buttressed by the highly successful clinical impact in melanoma, immunotherapy is gaining momentum as the next treatment modality for many human cancers. Chimeric antigen receptors (CAR) contain the antigen binding moieties of a monoclonal antibody and the co-stimulatory and signaling domains associated with effector receptor signaling. Bispecific antibodies (BsAb) combine the binding specificities of two different monoclonal antibodies, one activating a receptor on a killer effector cell, while the other engaging a tumor-associated antigen to initiate tumor cytotoxicity. In this review, we survey the HCC targets for which CARs and bispecific antibodies have been generated. The pros and cons of these targets for T-cell and Natural Killer cell based immunotherapy will be discussed.

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Introduction

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is the fifth most common cancer and the second most common cause of cancer deaths worldwide. HCC developed mostly in livers with chronic inflammation. The main causal factors for the latter are chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. However, other factors such as excessive alcohol consumption, non-alcoholic fatty liver disease, obesity, diabetes, aflatoxin, and smoking also play important roles in the pathogenesis of this neoplasm [12]. Despite advances in treatment, the five-year survival rate of patients with HCC remains poor averaging 5–15% [12]. Although viral vaccines [66] and effective anti-hepatitis drugs [72] in recent years have greatly reduced the incidence and the severity of chronic infection in developed countries, for the rest of the world, these benchmarks will take decades to be realized. In the meantime, finding an effective therapeutic for HCC remains an unmet need.

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Immunobiology of liver and liver cancer

Through the portal blood flow from intestine to heart, bacterial products, toxins and antigens continually challenge the liver parenchyma. To effectively neutralize these environmental threats, the liver contains a rich source of innate immune cell including macrophages, natural killer (NK) cells, NKT cells, and γδT cells. Among these, NK cells are a unique population comprising about 30–50% of liver lymphocytes in healthy individuals and up to 90% in liver malignancies [36]. Compared to the peripheral blood NK cells, liver NK cells are more cytotoxic against HCC. In fact, interleukin-2 (IL2)-stimulated liver NK cells express tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), whose receptor is highly expressed on poorly differentiated HCC [49]. Although this local population of NK cells generally have low CD16 expression [16], a limitation for most natural anti-tumor IgG1 antibodies, a BsAb targeting CD16 could potentially overcome this limitation. Since NK cells are less restricted by the immunosuppressive tumor microenvironment intended for T cells, their activation using bispecific antibodies against HCC is appealing.

The role of Lymphocytes in defense against HCC is well known. In fact, there is a positive correlation between T and NK cell infiltration into the tumor site and higher survival rate in HCC patients [23,50]. However, immunosuppressive tumor microenvironment

undermines lymphocyte function. Myeloid derived suppressor cells [38], mesenchymal stem cells [112], regulatory T cells [104], cancer-associated fibroblasts [3], tumor associated macrophages [28], and programmed cell death protein 1 (PD-1)^{hi} regulatory B cell [107] suppress immune cells and promote HCC progression. Furthermore, overexpression of inhibitory receptors including PD-1 and T cell immunoglobulin and mucin-domain containing-3 (TIM-3) on circulating or tumor-infiltrating T cells was associated with poor clinical outcomes [61,96]. In addition, PD-1 ligand (PD-L1) or B7-H3 expressed on HCC cells can induce T cell apoptosis or inhibit T cell functions [96,98]. Hence, modulation of immunosuppressive cells and molecules is an active area of investigation [51,61,103].

A Phase I/II clinical trial to evaluate the safety and efficacy of nivolumab, a fully human IgG4 PD-1 blocking monoclonal antibody, was completed on 41 patients with HCC. Patients were treated for up to two years with nivolumab (0.1–10 mg/kg intravenously) [29]. Among 39 patients whose response was evaluable, 2 complete responses, 7 partial responses, 18 stable diseases, and 12 progressive diseases were reported. 71% patients experienced drug-related adverse effects (17% grade 3/4) including rash and elevated serum levels of aspartate aminotransferase, alanine aminotransferase, and amylase. Overall survival rate was 72% after six months. In a recent case report, a 75-years old male patient with metastatic HCC unresponsive to sorafenib was treated with pembrolizumab, a humanized IgG4 PD-1 blocking monoclonal antibody. After six cycles of treatment (each cycle with 2 mg/kg every three weeks), the patient's tumor mass and blood alpha fetoprotein was markedly reduced [103]. In murine models of HCC, it was shown that sorafenib treatment increases intratumoral hypoxia leading to increased expression of stromal cell-derived 1 alpha (SDF1α), PD-1 ligand (PDL1), and accumulation of immunosuppressive cells [19]. Combination of sorafenib, murine PD-1 blocking antibody, and SDF1α receptor inhibitor provided the most potent tumor growth delay [20].

Antibody-based T cell-dependent immunotherapy

Compelled by the striking recent clinical results of T-cell based therapies, cancer immunotherapy was named as the breakthrough of the year in 2013 [24]. Chimeric antigen receptors (CAR) and bispecific antibodies are two powerful extensions of this approach. CARs were originally developed by fusing the antigen-binding moiety of an antibody to the transmembrane and cytoplasmic domains of CD3ζ [32]. T cells equipped with these CARs had in vitro cytotoxicity, but their in vivo persistence and function were suboptimal. Therefore, second and third generation CARs were developed by addition of one or two costimulatory domains (e.g. CD28, 4-1BB, OX40), respectively to the intracellular domain of the first generation CARs (Fig. 1) [74]. Bispecific antibodies (BsAb) combine the specificities of two monoclonal antibodies in a single molecule. These bispecific reagents can neutralize the effect of two tumor-associated-antigens. More commonly, they activate effector cells and bring them to engage cancer cells to execute their cytotoxic functions [74]. CAR technology has been tested against the target CD19 (in human cancers such as ALL, CLL), GD2 (neuroblastoma), CD22 (ALL), mesothelin (mesothelioma), and HER2 (sarcoma) [4,8,35,42,73,86], and IL13R (glioblastoma) [13] with overwhelming success in CD19(+) leukemia, but only select solid tumors. Several factors might be responsible for the inferior efficacy of CART cells in solid versus hematological malignancies including target antigen heterogeneity, poor trafficking and penetration of therapeutic cells, and the hostile tumor microenvironment (hypoxia, acidosis, nutrient depletion, tumor-derived immunosuppressive molecules, various immunosuppressive cells including tumor-associated macrophages, regulatory T cells, and myeloid-derived suppressor cells) [81]. BsAb was successfully proven for CD19 (ALL), leading to the FDA approval of blinatumomab for Philadelphia chromosome (ph)-negative relapsed/refractory B-cell precursor ALL (BLINCYTO™) in 2016. BsAb targeting CD33, CD52, HER2, CD20, GD2, GPC3, and CD123 are in the clinical pipeline. It is timely to review preclinical and clinical studies where these T-cell based therapeutic strategies have been applied to HCC. Table 1 summarizes the BsAb and CAR targets of HCC. Table 2 summarizes clinical trials involving anti-HCC BsAb and CARs. Tables 3 and 4 summarize the characteristics of BsAbs and CARs used against HCC targets.

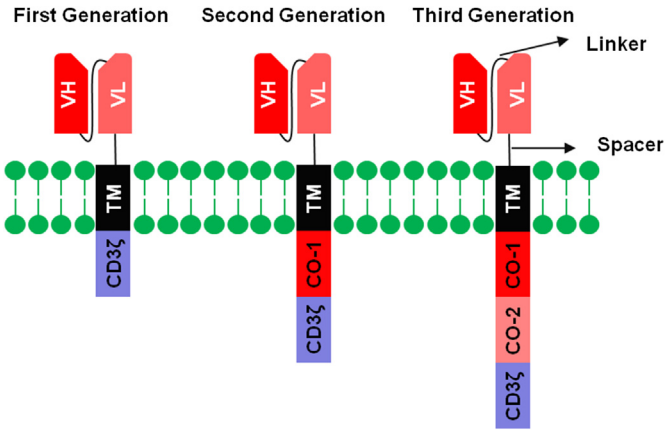

Fig. 1. Structure of chimeric antigen receptors (CAR). CARs are composed of a single-chain fragment variable (scFv, containing the heavy chain variable domain (VH) and the light chain variable domain (VL) of a monoclonal antibody attached together via a flexible linker) linked via a spacer sequence to a transmembrane (TM) domain and to the CD3ζ chain (first-generation CARs). Second- and third-generation CARs additionally contain one or two costimulatory domains, respectively. VH, heavy chain variable domain; VL, light chain variable domain; TM, transmembrane domain; co-1, costimulatory domain 1; co-2, costimulatory domain 2.

Glypican-3 (GPC3)

GPC3 (also known as GTR2-2, intestinal protein OCI-5, and MXR7) is a heparan sulfate proteoglycan expressed as a 70 kDa precursor protein. Upon cleavage by furin, GPC3 is divided into an N-terminal 40 kDa and a C-terminal 30 kDa fragment. The latter, which contains two heparin sulfate chains, is attached to the cell membrane via a glycosylphosphatidylinositol anchor [46].

It has been shown that GPC3 is expressed on the majority of hepatocellular carcinoma (HCC) and hepatoblatsoma cases but not or to a lower degree on normal liver tissue [48,54,64,80]. Interestingly, GPC3 expression could be used as an HCC precancerous marker in cirrhotic livers since it is associated with dysplasia in cirrhotic livers [71]. Furthermore, overexpression of GPC3 in HCC is associated with poor prognostic indicators including poor tumor differentiation, higher TNM score, and tumor invasion into blood vessels [62].

Glypican-3 CAR

The first GPC3 CAR was generated by fusing the scFv of an anti-GPC3 antibody to the CD8α hinge and transmembrane regions followed by the intracellular signaling domain of the CD3ζ. This first-generation CAR does not have the co-stimulatory domains. Therefore a third-generation CAR composed of the anti-GPC3 scFv, CD8α hinge, CD28 transmembrane and intracellular signaling domains, 4-1BB, and CD3ζ was produced. Although human T cells transduced with concentrated lentiviral vectors containing either of the CAR constructs specifically lysed HCC cell lines, the third-generation CAR T cells produced higher levels of interleukin-2 and IFNγ, which was positively correlated with the level of GPC3