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Advances of Immune Checkpoint Inhibitors in Tumor Immunotherapy

Qiao Guo

School of Life Sciences, Sun Yat-sen University, Guangzhou, China
Email: 609831965@qq.com

Abstract. Immune checkpoints are cell surface molecules that can fine-tune the immune responses, they are crucial for modulating the duration and amplitude of immune reactions while maintaining self-tolerance in order to minimize autoimmune responses. Numerous studies have demonstrated that tumors cells can directly express immune-checkpoint molecules, or induce many inhibitory molecules expression in the tumor microenvironment to inhibit the anti-tumor immunity. Releasing these brakes has emerged as an exciting strategy to cure cancer. In the past few years, clinical trials with therapeutic antibodies targeting to the checkpoint molecules CTLA-4 and PD-1 have rekindled the hope for cancer immunotherapy. In contrast to the conventional treatment, checkpoint inhibitors induce broad and durable antitumor responses. In the future, treatment may involve combination therapy to target different checkpoint molecules and stages of the adaptive immune responses. In this review, we summarized the recent advances of the study and development of other checkpoint molecules in tumor immunotherapy.

1. Introduction

The activation of T cells by antigen presenting cell is tightly controlled by the immune system. T cells are initially stimulated by the ligation of the T cell receptor (TCR) and the cognate major histocompatibility complex (MHC)/peptide molecules, this is also called “signal one”. Optimal T cell activation requires a “second signal” which is provided by co-stimulation molecules [1]. Many of these molecules belong to the tumour necrosis factor (TNF) family and B7 family. TNF family molecules bind to TNF receptor superfamily members, and mainly induce a positive signal [2]. All B7 family members belong to the immunoglobulin superfamily, which can bind both stimulatory and inhibitory receptors. The growing B7 family now comprises ten members, including CD80, CD86, PD-L1, B7-H3, B7-H4, Vista, etc [3]. Compelling evidence indicates that B7 molecules provide crucial positive or negative signals to control T-cell activation, they act as checkpoint that controls the threshold for whether a given TCR interaction leads to activation or anergy. They also play an important role in anti-tumor immunity [4, 5]. In 2011, FDA approved the CTLA-4 blocking antibody, Ipilimumab (BMS) for the treatment of advanced melanoma [6]. In 2014, FDA approved two PD-1 blocking antibodies, Pembrolizumab (Merck) and Nivolumab (BMS), for use in patients with advanced or unresectable melanoma who fail to respond to other therapies, which represent the beginning of a new era for cancer immunotherapy [7, 8].

2. Immune checkpoint inhibitors in developing

The therapeutic monoclonal antibodies targeting to immune checkpoint molecules represent a new, efficient alternative to the standard treatment of advanced cancers. However, the response rate is still low, 10% to 30% in most cases, suggesting strategies targeting to other checkpoint molecules or combinatorial treatment is necessary [9, 10].

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2.1. Tim-3

Tim-3 consists of extracellular IgV domain and mucin domain, a transmembrane domain and intracellular domain. Tim-3 doesn't have a classical inhibitory signaling motif such as ITIM and ITSM in its intracellular domain, but has five tyrosine residues which can be phosphorylated by kinases [11]. The C-type lectin galectin-9 is identified as a Tim-3 ligand. Binding of galectin-9 to Tim-3 triggers the phosphorylation of the tyrosine residues in its cytoplasmic tail, which then recruits various downstream signaling molecules. Galectin-9 induces cell death in Tim-3+ Th1 cells and ameliorates EAE in a Tim-3 dependent manner [12]. So, Tim-3 plays an inhibitory role in regulating immune responses. Other ligands, such as Ceacam-1 and HMGB-1 are also reported to bind to Tim-3 [13].

Tim-3 may be a key checkpoint molecule in suppressing the anti-tumor immunity. In mice tumor model, it is demonstrated that Tim-3 marks the most dysfunctional cell subset of CD8+ T cells in the tumor infiltrating lymphocytes. All of the CD8+ Tim-3+ T cells co-express PD-1, and these PD-1, Tim-3 double positive CD8 T cells exhibit more severe dysfunctional phenotype in both proliferation and effector cytokines production [14]. In patients with non–small cell lung cancer (NSCLC) or advanced melanoma, it is found that about one third of CD8+ tumor infiltrating lymphocytes express Tim-3. Meanwhile, in patients with NSCLC, Tim-3 is expressed in 60% CD4+FoxP3+ TILs, but not in PBMCs. And the Tim-3+ Tregs correlates with the nodal metastases and advanced cancer stage. Tim-3+ Tregs is also found in many other cancer types, such as hepatocellular, ovarian, colon, and cervical carcinomas [15].

2.2. LAG-3

Lymphocyte activation gene-3 (Lag-3) is mainly expressed on effector CD4+ and CD8+ T cells, Treg and a subset of natural killer (NK) cells. Like Tim-3, LAG-3 also does not contain ITIM and ITSM motifs in its cytoplasmic domain but has three unique regions. The first region contains a serine-phosphorylation site, the second one contains a KIEELE motif, and the third one contains glutamic acid-proline (EP) repeats [16]. LAG-3 resembles the CD4 molecule structurally, and can bind to MHC class II with an affinity even higher than CD4 [17]. LSECtin and Galectin-3 are also identified as LAG-3's ligand. LSECtin belongs to the DC-SIGN family, is firstly found to be expressed in the liver cells and can help to protect the hepatocytes from HBV infection. Tumor cells also express LSECtin that functions to suppress tumor-specific T cell immune responses [18]. Galectin-3 is mainly expressed on tumor-infiltrating CD8+ T cells and inhibits anti-tumor T cell reactions in a LAG-3-dependent way [15]. It is demonstrated that Lag-3 negatively regulates T cell expansion and can promote Treg cell mediated immune suppression. Administration of superantigens Staphylococcal enterotoxin B (SEB) and OVA in Lag-3 deletion mice results in uncontrolled proliferation of CD8+ and CD4+ T cells [19]. Meanwhile, blockade of Lag-3 on Treg cells abrogates Treg cell's suppressive functions whereas ectopic overexpression of Lag-3 in non-Treg CD4+ T cells infers a Treg-like phenotype.

Lag-3 is highly expressed on CD4+ and CD8+ tumor infiltrating lymphocytes (TILs) in both murine tumor models and several cancer types in human, such as melanoma, ovarian cancer and colorectal cancer. Lag-3 expression is also found to correlate closely with PD-1 on exhausted CD8+ T cells. Although blockade of Lag-3 alone had little effect, blockade of Lag-3 synergized with PD-L1 has been shown to improve the anti-tumor immune responses [20]. For example, dual blockade of Lag-3 and PD-L1 greatly improves the proliferation and cytokine production of tumor-antigen-specific CD8+ T cells. Lag-3 blockade is also proven to synergize with tumor vaccine to improve tumor-specific CD8+ T cell reactivity. Antibodies that targeting to Lag-3 are now being explored in the clinic. These trials are exploring the use of anti-Lag-3 antibodies alone or in combination with anti-PD-1 in different solid tumors [21].

2.3. B7-H3

B7-H3, also known as CD276, is a type I transmembrane protein that belongs to the B7 family. The extracellular domain of murine B7-H3 contains one IgV and one IgC domains, while human B7-H3 has two isoforms, with the longer one containing a tandem repeat of IgV and IgC domains [22]. The receptor of B7-H3 is still unknown, though it is reported that its receptor can be induced upon T cell stimulated with PHA or ConA. Data shows that B7-H3 provides inhibitory signals in regulating

immune reactions. In the experimental autoimmune encephalomyelitis (EAE) disease model, B7-H3 deletion mice generate higher amount of autoantibodies to DNA and phenotypes appear several days earlier than the wildtype littermates [23]. Moreover, it was shown that B7-H3 negatively regulates Th2-mediated immune responses.

Recent studies found aberrant B7-H3 expression on a wide variety of cancers. In non-small cell lung cancer, pancreas, liver, kidney, breast and ovary cancer, the expression of B7-H3 is correlated with advanced disease and poor prognosis [24, 25]. In contrast to the above cancer types, tumor-associated B7-H3 expression in patients with gastric carcinoma and pancreatic cancer was found positively related with the survival time. So, the role of human B7-H3 in anti-tumor immunity is controversial. It might be due to different isoform expression in different cancers or B7-H3 might have more than one receptors, that some with costimulatory functions while the other with coinhibitory functions [26]. Thus, the exact functions of B7-H3 as immune modulator still needs to be further explored.

Now, about 10 clinical trials that targeting to B7-H3 are being carried out in different cancers. The preliminary results show that B7-H3 is a promising target for cancer immunotherapy to the B7-H3-expressing tumors. But considering the complicated expression pattern and controversial functions of B7-H3 in different cancers, it should be very cautious to analyze the clinical results.

2.4. B7S1

B7S1, also known as B7-H4 and B7x, is another B7 family member [27]. B7S1 mRNA transcript is widely distributed in various peripheral tissues, while the cell membrane B7S1 protein is very rare. Fresh isolated lymphocytes such as T cells, B cells and monocytes do not express B7S1 protein, but B7S1 surface expression is induced upon stimulation by LPS and IFNγ [28]. The receptor of B7S1 is still need to be identified, though its putative receptor is reported to be expressed on activated T cells. It was demonstrated that B7S1 plays an inhibitory role on the T cell growth, cytokine secretion, and development of cytotoxicity function. B7S1 gene deletion mice showed augmented Th1 cell responses. Blocking antibody that targeting to B7S1 significantly enhance T cell proliferation in vitro and exacerbates EAE in vivo [27].

B7S1 is expressed in a wide variety of tumors. Numerous studies report the association of intra-tumor B7S1 expression with poor prognosis. It was shown that the death rate of renal cell cancer (RCC) patients with tumor cells expressing B7S1 is three times higher than patients with tumor lacking B7S1 expression [29]. In addition to RCC, B7S1 expression in many other tumors, including colorectal, NSCLC, prostate, and ovarian, also closely correlated with disease stage and cancer progression. Thus, all these evidences indicated that B7S1 protein is a potential prognostic marker for patients with malignant tumors [24].

Although the functions of B7S1 in tumor immunity has not been fully understood and no clinical trial targeting to B7S1 is ongoing, some pre-clinical studies demonstrates it is a promising target for cancer immunotherapy. In 2014, Jeon and colleagues developed a therapeutic monoclonal antibody against B7S1 and found that it significantly inhibited the growth of B7S1-expressing tumors in vivo [30].

2.5. VISTA

VISTA, also known as PD-1H, is identified by two groups independently in 2011 [31, 32]. The extracellular domain of VISTA bears significant homology with PD-L1. VISTA is mainly expressed in hematopoietic cells with the highest expression level on myeloid derived cell, such as dendritic cells, macrophages, and lower level on B cells and T cells. It is also reported that in human PBMC, VISTA expression closely correlated with CD11b expression [32].

VISTA is a negative immune checkpoint molecule that suppresses T cell activation. The development of lymphocytes in young Vista-deficient mice is normal, but the spontaneously activated T cells gradual accumulates in older Vista knockout mice, accompanied by production of a spectrum of inflammatory cytokines and chemokines.

VISTA/PD-1 double deletion mice mount a higher magnitude of T-cell response toward foreign antigens than the single gene deletion mice, implying that VISTA and PD-1 regulate T-cell responses in a non-redundant manner [33].

The role of VISTA in anti-tumor immunity is under active investigation. VISTA-expressing MCA105 fibrosarcoma grows rapidly in vaccinated hosts, whereas the wildtype MCA105 fibrosarcoma lacking VISTA expression fails to thrive [32]. In a murine brain glioma model, VISTA knockout mice are highly resistant to tumor cell inoculation [34]. Now several clinical trials are being carried out in solid tumors. In the clinical trial NCT02812875, CA-170 is a small chemical molecule that directly targets the PDL1, PDL2 and VISTA immune checkpoints and results in activation of T cell proliferation and cytokine production. Now, this trial is on the recruiting stage.

3. Concluding Remarks

Immune checkpoint immunotherapies are proven to elicit durable control of cancer progression and even complete remission in patients that don't respond to other treatments. The administration of checkpoint blocking antibodies reverses "cold" tumor microenvironment into a "hot" one, and enhances the anti-tumor immunity activities [35]. As summarized above, the function of these five checkpoint molecules in tumor immunotherapy is under active investigation, they can be used alone or in combination with CTLA-4 or PD1 blocking antibody [36]. Meanwhile, the "personalized" treatment that based on high throughput sequencing of the genome of an individual's cancer cells to identify specific mutations in genes is developing quickly, the neoantigen vaccine therapy is also a very promising method [37, 38]. The combination of checkpoint inhibitor with neoantigen vaccine is considered to be an optimized treatment to cancers with high mutation burden.

4. References

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