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Research on the Mechanism and Clinical Application of CTLA-4 in Tumor Immunotherapy

Xiaochen Sui

Clinical Medicine, West China School Of Medicine, Chengdu, Sichuan, 610041, China

ABSTRACT
Immune checkpoint inhibitors, as an important therapeutic approach, are playing an increasingly important role in current cancer treatment, providing newer and better options compared to traditional tumor treatment regimens. Clarifying its mechanism of action and clinical application is the key to improving therapeutic effect and prognosis. Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) plays a key role in regulating the proliferation and activation of T cells. The CTLA-4 molecule competitively binds to the B7 molecule with CD28, affecting the kinetics of T-cell responses. In regulatory T cells (Tregs), the stimulation of T-cell receptor (TCR) up-regulates the expression of CTLA-4 to inhibit antigen-presenting cells (APCs). The review will summarize the molecular mechanism and immunosuppressive mechanism of CTLA-4 immune regulation, as well as the research and development and clinical application of CTLA-4 inhibitors.

KEYWORDS
Cytotoxic T lymphocyte-associated protein 4 (CTLA-4); Immune checkpoint inhibitors (ICIs); Bispecific antibodies (BsAbs); Immune-related adverse events (IrAEs)

1 Introduction

Cancer remains a major global health challenge [1]. Traditional treatment methods, such as surgery, chemotherapy and radiotherapy, have limited therapeutic effects on cancer. Immunotherapy, a promising treatment approach in cancer therapy, has aroused great interest among scientists by potentiating the direct tumoricidal activity of T cells. CTLA-4 is currently a hot topic in immunotherapy research.

CTLA-4 is an immunoglobulin found on activated T cells, Tregs, and certain tumors. By competing with CD28 for B7 ligands, it reduces CD28-mediated signaling, cytokine production, and T cell proliferation. [6]By blocking CTLA-4, the checkpoint blockade relieves functional inhibition on T cells, thereby enhancing their anti-tumor cytotoxicity. Atkins et al. 's research confirmed that blocking this checkpoint can significantly improve the survival rates of patients with various cancers [2].

This review summarizes the mechanism of CTLA-4 in tumor immunotherapy, alongside the development, clinical application, and immune-related adverse events (irAEs) of its inhibitors.

2 The molecular mechanism and expression regulation of CTLA-4

CTLA-4, an immunoglobulin, is primarily found on activated naive T cells and FoxP3 Tregs, with additional expression on activated Teff cells and certain tumor populations. CTLA-4 is mainly located within cell vesicles and is expressed as a homodimer. It is transported to the cell surface through TCR/CD28 co-stimulation. Since CTLA-4 needs to be degraded or recycled through endocytosis mediated by mesh proteins, its expression on the cell membrane is unstable [3].

When TCR binds to the antigen, the expression of CTLA-4 in CD4+ and CD8+ T cells is rapidly upregulated and transported to the immune synapse. Its transfer volume is directly proportional to the TCR signal strength. Through competitive high-affinity binding to CD80, CTLA-4 accumulates stably at the synapse, inhibiting CD28's engagement with CD80/CD86 and its subsequent PI3K-AKT signaling. It is worth noting that the intracellular segment of CTLA-4 itself can also recruit PI3K, which underactivates AKT under non-response conditions, promoting T cell survival by inactivating BAD and upregulating Bcl-xL, which instead helps maintain immune tolerance. In Tregs, CTLA-4 is continuously expressed, and its transcription is controlled by Foxp3. TCR engagement leads to the upregulation of CTLA-4 [4].

3 The immunosuppressive mechanism of CTLA-4

T cell activation necessitates two stimuli. Signal 1 originates from TCR-pMHC interaction, and Signal 2 from CD28 binding to B7 molecules on antigen-presenting cells (APCs). The CD28 signal sustains T cell survival, stimulates cytokine secretion, and ultimately facilitates clonal proliferation and differentiation [4].

3.1 Compete with CD28 for the B7 ligand

By virtue of its superior affinity for shared B7 ligands, CTLA-4 effectively antagonizes CD28 binding and suppresses the resulting T cell activation signals. CTLA-4 competitively binds B7 ligands, thereby antagonizing CD28-mediated co-stimulation and suppressing T cell activation. This inhibitory mechanism involves the reduction of key cytokines and the attenuation of T cell proliferative capacity. Furthermore, CTLA-4 engagement with B7 molecules on APCs can lead to functional suppression of APC activity, thereby reinforcing immune downregulation [3,7].

3.2 Inhibit T cell activation via IDO upregulation

CTLA-4 engagement of CD80/CD86 enhances the expression of indoleamine 2,3-dioxygenase (IDO), which facilitates tumor immune escape by catalyzing tryptophan breakdown. IDO exerts its immunosuppressive effects through dual mechanisms: on one hand, it depletes local tryptophan necessary for T cell function and accumulates pro-apoptotic kynurenine; on the other hand, it drives the generation of CD4+CD25+FoxP3+ Tregs from naive precursors, ultimately suppressing T cell activation [5].

3.3 Disrupt T cell-APC contact

CTLA-4 suppresses T cell activation through multiple mechanisms: it promotes T cell motility, which curtails contact duration with APCs and lowers major histocompatibility complex (MHC) presentation efficiency, thereby raising the activation threshold and preventing autoimmunity [5].

3.4 Affect the formation of immune synapses

CTLA-4 additionally suppresses T cell activation by inhibiting the expression of lipid rafts—sphingolipid-rich microdomains essential for immune synapse assembly—and by disrupting the formation of TCR-signaling microclusters [6].

3.5 Up-regulate the expression of CTLA-4 in Tregs

In Tregs, CTLA-4 mediates co-stimulatory blockade by downregulating CD80/CD86 expression on APCs, thus restricting CD28-mediated activation of naïve T cells; moreover, it can drive trans-endocytosis to internalize and degrade B7 molecules from the APC surface. Moreover, Tregs can produce a large amount of a selective splicing soluble CTLA-4 subtype that can bind to the B7 molecule and inhibit T cell responses through exogenous mechanisms [4].

4 Research and development and clinical application of CTLA-4 inhibitors

The inhibitory function of CTLA-4 on T-cell responses establishes it as a prominent immune regulatory target. Its inhibitors (such as Yervoy and tremelimumab) can exert an immune-stimulating effect by blocking this pathway and relieving the inhibition [7].

4.1 The mechanism of action of CTLA-4 inhibitors

Ipilimumab and teremimumab are typical CTLA-4 inhibitors, among which only the former has been approved by the FDA and EMA. The anti-tumor efficacy of CTLA-4 inhibition is mediated by a sustained anti-cancer immune response. This involves not only the potentiation of effector T cell activity but also the antibody-dependent cell-mediated cytotoxicity（ADCC）-mediated depletion of immunosuppressive Tregs within the tumor. Furthermore, given that CTLA-4 can also be expressed in tumor cells, IgG1-based anti-CTLA-4 monoclonal antibodies (such as ipilimumab) may be able to directly lyse tumor cells through FC-mediated ADCC effects. A correlation has been observed between elevated CTLA-4 expression in melanoma tumors and an improved clinical response to ipilimumab. In the tumor cell attack experiment, animals treated with anti-CTLA-4 could rapidly clear cancer cells, which confirmed that the inhibition of CTLA-4 could induce long-lasting immune memory [9].

4.2 CTLA-4 inhibitors combined with other immune checkpoint inhibitors

Due to the limited efficacy of ipilimumab monotherapy (only some patients benefit sustainably), its subsequent approved indications (including melanoma itself) are all for combination with the anti-PD-1 monoclonal antibody. This has demonstrated superior efficacy over traditional treatments (chemotherapy, targeted drugs) or single-agent immunotherapy in various tumors such as melanoma, RCC, NSCLC, and MPM, significantly improving the objective

response rate (ORR), progression-free survival (PFS), and overall survival (OS), with some patients achieving long-term survival [8].

The currently commonly used combination therapy of dual immune checkpoint inhibitors (ICI), which simultaneously blocks CTLA-4 (the early T cell activation stage) and PD-1/PD-L1 (the effector T cell reactivation stage), collaboratively enhances the anti-tumor immune response.

4.3 The mechanism of action and clinical application of bispecific antibodies

There are currently two main problems with combined therapy. Firstly, irAEs are often observed during the use of combined ICI such as ipilimumab and nivolumab. Secondly, when using ICI, T-cell receptors have MHC limitations, which can easily lead to immune escape. A promising solution is to use bispecific antibodies (BsAbs) to achieve dual-antigen co-blocking in the Tumor microenvironment(TME), that is, by confining the immune response to the local tumor area, thereby reducing its expected toxicity to normal tissues [9].

BsAbs exert their anti-tumor functions in a synergistic way by simultaneously binding to two different antigens, redirecting immune cells, regulating signaling pathways (including activating co-stimulatory signals and blocking inhibitory signals), and combining targeted antigens. One advantage of BsAbs is that they target tumor cells more precisely, aiming at dual tumor-associated antigens (TAAs), specifically binding to cancer cells expressing both antigens, confining their action to circulating tumor cells, the TME, and metastatic sites, while avoiding targeting single-positive healthy cells [9].

BsAbs targeting CTLA-4 represent a novel type of immunotherapy. For example, the bispecific antibody AT0R-1015 can bind to CTLA-4 and the co-stimulatory receptor OX-40 respectively. By simultaneously targeting these two immune checkpoints, it can, on the one hand, regulate immune balance and, on the other hand, enhance the activation and proliferation of effector T cells, thereby improving the killing effect on colon, pancreatic and bladder cancer cells. Through simultaneous blockade of CTLA-4 and PD-1, BsAbs achieve synergistic immune activation: they potentiate effector T cells, attenuate Treg-mediated suppression, and boost cytotoxic killing. This integrated approach not only generates a more robust and lasting anti-tumor response but also, by confining the action, may mitigate the systemic toxicities linked to broad Treg loss in normal tissues [10].

Both preclinical and clinical studies have confirmed that the dual blocking of CTLA-4 and PD-1 can produce a synergistic anti-tumor effect. This strategy not only significantly enhanced the therapeutic effect in tumor models, manifested as an upregulation of the effector T cell/suppressor cell ratio and the secretion of pro-inflammatory factors, but also outperformed monotherapy in clinical applications such as melanoma and renal cell carcinoma. In patients with melanoma, this combined therapy can stimulate stronger specific cellular immunity, such as enhanced T cell frequency and function as well as dynamic changes in B cells [10].

There are relevant literature reports in lung cancer that the efficacy of ICI is significantly improved compared with BsAbs, which simultaneously target two immune checkpoints through a single molecule, especially for patients with high TMB or positive specific biomarkers [11].

5 Clinical challenges and immune-related adverse events

Immune-related adverse events (IrAEs) remain the main challenge in current tumor immunotherapy. CTLA-4 blockade (especially in combination with PD-1 blockade) significantly increases the incidence and severity of severe irAEs (such as colitis, hepatitis, endocrine diseases, rashes, pneumonia, etc.). High-dose ipilimumab (10mg/kg) is more toxic. IrAEs require close monitoring and timely management with immunosuppressants such as glucocorticoids, which may lead to treatment interruption [12].

6 Conclusion and Future Directions

CTLA-4 inhibitors are the cornerstone of cancer immunotherapy. Among them, ipilimumab, as a first-in-class drug, has for the first time successfully improved the survival period of patients with melanoma by blocking immune checkpoints. Although dual CTLA-4/PD-1 blockade significantly improved the therapeutic effect, toxicity limited its wider application. The novel CTLA-4 targeted drugs that specifically activate (proantibodies) in the TME or selectively target tumor-infiltrating lymphocytes (bispecific antibodies) co-expressing immune checkpoints is a promising direction for overcoming toxicity, further improving the therapeutic index and expanding the beneficiary patient population.

Though BsAbs have low-affinity off-target binding, they cannot effectively induce ADCC after Fc engineering modification, and thus are expected to avoid Tregs that depletes normal tissues to reduce immune-related adverse

reactions. Furthermore, this dual blocking strategy can also counteract the excessive activation of Tregs in the TME due to the inhibition of PD-1 monotherapy.

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