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Targeting CTLA-4 in Cancer: Biological Insights with a Focus on Renal Cell Carcinoma

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Abstract

Renal cell carcinoma (RCC) is a complex group of malignant tumors characterized by immunosuppression and high invasiveness. In the majority of patients with advanced renal cell carcinoma, treatment fails to achieve a complete cure post-treatment. Efforts are needed to develop new therapeutics to improve the outcome of renal cell carcinoma. The "immune checkpoint" of T cells has attracted much attention in tumor immunotherapy. It is widely accepted that suppressor T cell immune checkpoints promote tumor immune escape through negative immune regulatory signals (cytotoxic T lymphocyte associated antigen 4 [CTLA-4], programmed cell death 1 [PD-1], B7-H3, and B7-H4, among others). The current data suggest that the PD-1 and CTLA-4 receptors inhibit the T cell receptor and its proliferation. Blockade of the PD-I/PD-L1 and/or CTLA-4/CD 28 pathways has shown favorable tumor outcomes in clinical trials in advance-stage renal cancer. This article reviews the role of CTLA-4/CD 28 pathway in renal cell carcinoma. Here we discuss the basics of the CTLA-4 pathway from a physiological perspective and evaluate the results of clinical studies of CTLA-4 alone and in combination with PD-1/PD-L1 blockers to support future studies of combination immunotherapy.

Key words immune checkpoint inhibitors, renal cell carcinoma, anti-CTLA-4, biological insights

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Introduction

Kidney cancer is one of the 10 most common cancers in men and women, accounting for 5% and 3% of all malignancies [1], exhibiting heterogeneous and complex phenotypes [2]. Among them, renal cell carcinoma (RCC) is the most common form of renal cancer, accounting for 85% of cases with higher males to female ratio (1.7:1) [3, 4]. At the time of diagnosis, an estimated 25% of patients will have metastatic disease. Approximately 30% of patients who undergo nephrectomy will still develop metastatic disease [5]. Based on data from 2008 to 2014, the 5-year survival rate for localized tumors is 93%, but for metastatic kidney cancer it is 12%. Observational studies have found that the median survival of patients treated with targeted therapies improves to approximately 40 months, with progression-free survival (PFS) as high as 27 months for some therapies, leading to the widespread use of these agents in RCC [6]. However, the 5-year survival rate of patients with metastatic disease is still very low, especially in patients with poor prognostic factors [7]. Therefore, new therapies are needed to improve the prognosis of patients with advanced tumors.

The ability to evade immune surveillance and programmed cell death is a major mechanism for evasion of cancer [8]. Various tumors may express biomarkers that prevent the host from generating an immune response [9]. In fact, this is essential for that host's regulation of cell proliferation to prevent damage to the nuclear content. It is well known that cell division halts after sustained cell damage to allow repair. When repair is not possible, cell death is induced to prevent the development of defective cell. The rare cases of spontaneous response of RCC provide information that the immune system may be able to suppress RCC through antitumor immunity [10]. Immunotherapy is an increasingly popular and researched treatment that uses the body's own immune system to fight metastatic cancer [9]. Immune checkpoint inhibitors (ICI) upregulate the immune response by blocking programmed cell death protein 1 (PD-1) receptors, ligands of PD-1, or cytotoxic T lymphocyte-associated protein 4 T cells, leading to a new era of immunotherapy [11]. CTLA-4 and PD-1 are inhibitory receptors with molecular significance. A large number of these agents have been developed, studied, and are currently marketed as effective therapies for the treatment of mRCC and many other malignancies. This review describes CTLA-4 in detail.

Biology of Immune Checkpoint Inhibition

Immune Checkpoint Inhibition (ICI) is a cancer immunotherapy that enhances the anticancer immune response by targeting immune receptors on the surface of T lymphocytes [12]. Considered a novel option for cancer treatment, these agents have a low toxicity profile in some cases and can achieve durable results [13]. In contrast to traditional therapeutic strategies, ICI acts against tumor cells by reactivating the host immune system [13]. Immune checkpoints maintain a balance between pro-inflammatory and anti-inflammatory signals under steady-state conditions [13].

T cell activation is a complex process involving multiple stimulatory and inhibitory receptors. The initial step requires antigen-specific T cells to recognize MHC peptides, but also requires costimulatory signals from the interaction between membrane proteins on antigen presenting cells, including B7 family members CD 80 (also known as B7-1) and CD 86 (also known as B7-2), and CD28 on T cells [14-16]. A variety of signals influence the activation status of T cells, but cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1), and programmed cell death 1 ligand 1 (PD-L1) are the only immune checkpoints that currently have routine clinical application in patients with RCC [17, 18]. In addition, the biological and clinical significance of several other B7 family members is only now being elucidated [17].

Cytotoxic T Lymphocyte Antigen 4 (CTLA-4) biology

CTLA-4 (cluster of differentiation 152, CD152), also known as CD152, is typically located in the cytoplasm of CD4+ and CD8+ T cells, is induced on the cell surface, and binds CD80 and CD86 with higher affinity than CD28 [19, 20]. It was discovered in 1987 by Brunet et al [21] by screening a cDNA library derived from mouse cytolytic T cells. CTLA-4 expression is usually noted upon T cell activation, but regulatory T cells (Tregs) constitutively express CTLA-4 due to the high levels of the forkhead transcription factor FoxP3 [21-23]. CTLA-4 acts mainly by competing with CD28 receptor for binding to B7 ligands (B7-1/CD80 and B7-2/CD86) on antigen presenting cells (APC) [24]. During T cell activation, the CD28 receptor on the T cell binds to the B7 ligand on the APC and provides the necessary second activation signal for the T cell. However, the CTLA-4 receptor binds to B7 ligands with higher affinity and lower surface density, thus outperforming the CD28 receptor in binding to the B7 ligands. Thus, the absence of a second activation signal in the presence of the CTLA-4 receptor results in T cell anergy [20, 25, 26]. In addition, CTLA-4 receptor has also been shown to sequester B7 ligand from the APC surface and result in significant depletion of its surface ligand. Interestingly, due to its structural similarity to CD28 and its expression on activated T cells, CTLA-4 was considered to be a positive regulator of T cells in the first days of its discovery (**Figure 1**).

The fact that CTLA-4 acts on the cell surface suggests a strategy to enhance T cell immunity by using CTLA-4 inhibitory antibodies. Allison et al. has demonstrated the negative effects of CTLA-4 and established the antagonistic effects of CTLA-4 and CD28 on T cell stimulation. The study showed in detail that binding of CTLA-4 to B7 ligand abolished IL-2 secretion by T cells and T cell proliferation following TCR activation. Blocking CTLA-4 with anti-CTLA-4 antibodies leads to rejection of pre-established tumors, and mice lacking the Ctla4 gene (Ctla4-/- mice) develop severe lymphoproliferative and lethal autoimmune phenotypes [27-29]. Further studies have shown that CTLA-4 is involved in activating an intrinsic signaling cascade in T cells. It has been reported [30-34] that CTLA-4 activation inhibits IL-2 production and T cell proliferation and induces cell cycle arrest through interaction with pathways regulating cell survival and proliferation, including PI3K, NF-κB, and MAPK pathways. In addition, the interaction of anti-CTLA-4 monoclonal antibodies with CTLA-4 may activate antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-mediated lysis. We found that complement activation was lower in patients receiving ipilimumab (IgG1 antibody) than in patients receiving tremelimumab (IgG2 antibody) [35, 36]. Ipilimumab isotype IgG1 has a high affinity for FcγRIIIa, the Fc receptor mediating ADCC. More interestingly, patients with polymorphisms in this receptor were more responsive to ipilimumab [37].

Multiple signals, both stimulatory and inhibitory, modulate the activation of T cells by tumour cells or antigen-presenting cells. Tumour peptides are presented by major histocompatibility complex (MHC) glycoproteins and recognized by antigen-specific T cells. CTLA-4 acts as a negative regulator of T cell activation by binding to B7 ligands CD80 and CD86 expressed on antigen-presenting cells, thereby preventing the co-stimulatory interaction between CD28 and the B7 ligands. Ipilimumab binds to CTLA-4 and blocks the inhibitory signaling of the CTLA-4: B7interaction. PD-1 acts as a negative regulator of T cell activity predominantly

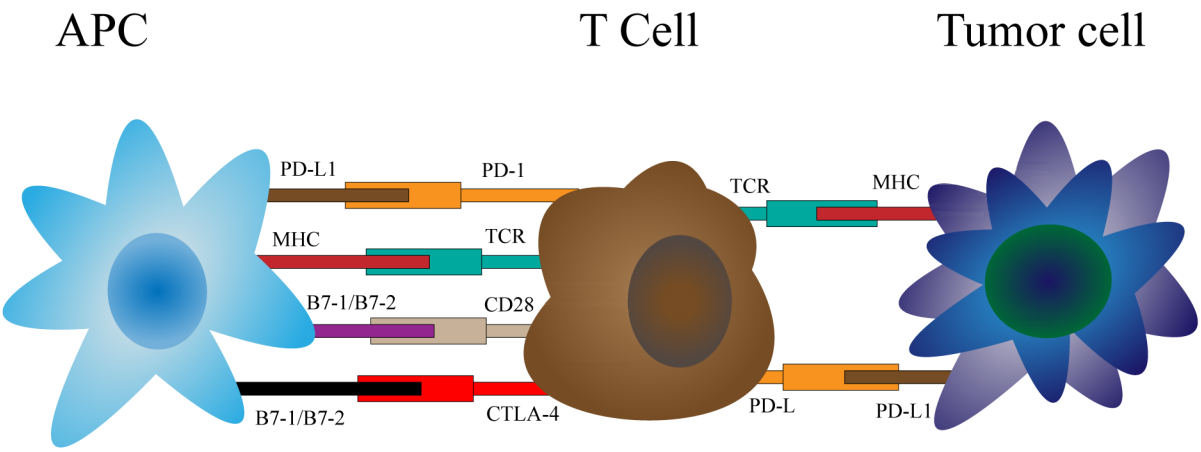

Figure 1. Mechanisms of action of CTLA-4.

by binding to PD-L1 on either tumor cells or antigen-presenting cells, leading to downstream signaling that inhibits anti-tumor T cell responses.

CTLA-4 expression in RCC

RCC is essentially a metabolic disease characterized by reprogramming of energy metabolism, and many of the genes mutated in RCC encode proteins that play a role in regulating cellular processes of oxygen and glucose consumption [38]. In particular, the metabolic flux is distributed through glycolysis [39-42], Mitochondrial bioenergetics and oxidative phosphorylation are impaired during lipid metabolism [40, 43, 44]. In addition, RCC is one of the most immunoinvasive tumors [45, 46]. Emerging evidence suggests that activation of specific metabolic pathways has a role in regulating angiogenic and inflammatory characteristics [47, 48]. VHL mutations in mRCC increase the transcriptional activity of its target genes (e.g VEGF, glucose transporter 1, and erythropoietin), independent of oxygen levels, promoting angiogenesis and immunosuppression [38]. The complexity of cell interactions and the depletion of available nutrients may create a nutrient-competitive environment for T cells and accumulate waste products that may damage T cells [49]. Rcc-bm exhibits metabolic changes resulting in altered pathways related to energy metabolism and oxidative stress, as well as accumulation of immunosuppressive metabolites such as tryptophan (TRP) [38, 49]. The enhanced activity of a series of interconnected oncogenic signaling networks centered on the PI3K-AKT pathway represents a generalizable feature in different BM histologies [49]. CTLA4 inhibits CD28 signaling and PI3K/Akt/mTORC1 signaling, resulting in decreased glycolysis and mitochondrial oxidative capacity [50]. Blocking the negative regulators of PD-1 and CTLA4, which impair CD28 signaling to inhibit T cell release, favors antitumor activity [38].

By survival analysis, it was found that high TII score had better prognosis than low TII score. GSEA analysis showed that the genes in high TII score group were rich in immunosuppressive pathways, such as ERBB signaling pathway, MAKP signaling pathway, mTOR signaling pathway and TGFβ signaling pathway [51]. A large number of clinical studies have confirmed that Anti-PD-1 and Anti-CTLA-4 antibodies can effectively inhibit the immune escape of cancer cells. Different from radiotherapy and chemotherapy drugs, the mechanism of immunotherapy is not to kill cancer cells directly, but to attack cancer cells indirectly by enhancing the specific anti-tumor cells of DC-CIK cells. Experimental study confirmed that the combination therapy can provide the synergistic anti-tumor effect of DC-CIK cells by suppressing proliferation, differentiation and early activation of RCC cells and regulating the immune stimulation and inhibiting the secretion of cytokines [52]. In RCC, about 1% of TIMCs express CTLA-4 [53] and the expression increased with the increase of tumor stage. In papillary RCC, up to 2.7% of TIMCs expressed CTLA-4 [54]. Polymorphisms in the CTLA-4 gene are associated with a higher risk of advanced ccRCC [55]. Allison and co-workers first demonstrated that administration of a CTLA-4 blocking antibody in mice prevented tumor establishment and induced rejection of established tumors [28].

CTLA-4 clinical trials in RCC

Ipilimumab

Ipilimumab is a human IgG1 mAb that can inhibit the function of CTLA-4 and was first approved and recommended for the treatment of melanoma in 2011 [56]. It selectively blocks the interaction between the negative regulation of CTLA-4 on activated T cells and its ligands CD80/CD86 expressed on immune cells. In a phase 2 trial (NCT 00057889), the efficacy of ipilimumab monotherapy was evaluated in patients with metastatic RCC disease treated with ipilimumab high-dose (HD) (3 mg/kg every 3 weeks) versus low-dose (LD) (3 mg/kg once followed by 1 mg/kg every 3 weeks) [57]. Of the 40 patients treated with HD ipilimumab, 5 achieved PR (12.5%), including patients who had previously progressed on IL-2 therapy [57]. Of the 21 patients with LD, only 1 had PR.no CRs in either arm. Grade 3 or higher TRAEs (Treatment-related Adverse Events), particularly autoimmune-related enteritis and endocrine defects, were present in 33% of patients. The most common autoimmune events were enterocolitis

Table 1. Immune checkpoint inhibitor-based adjuvant therapy trials in RCC.

Study	N	Description	Primary Outcome to be Assessed
NCT 00057889	61	Two sequential cohorts received either 3 mg/kg followed by 1 mg/kg or all doses at 3 mg/kg every 3 weeks	A primary end point of response by Response Evaluation Criteria in Solid Tumors (RECIST) criteria
NCT00372853	28	Tremelimumab (6 mg/kg, 10 mg/kg, or 15 mg/kg) intravenously once every 12 weeks and oral sunitinib (50 mg daily for 4 weeks then 2 weeks off or 37.5 mg daily as a continuous dose)	The primary objective was to determine the maximum tolerated dose (MTD). Secondary objectives were to assess antitumor activity, safety, and pharmacokinetics
NCT 02210117	1096	Nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks for four doses followed by nivolumab 3 mg/kg every 2 weeks, or sunitinib 50 mg/day for 4 weeks of each 6-week cycle	According to IMDC criteria, OS, PFS, and ORR in intermediate and low risk patients, ORR, PFS, and OS in the favorable risk group, and incidence of adverse events (AEs) in patients were secondary endpoints

(18%), hypophysitis (7%), and dermatitis (4%). No further studies have been conducted with ipilimumab monotherapy in RCC.

Tremelimumab

Tremelimumab is another anti-CTLA-4 monoclonal antibody. In phase 1 trial (NCT00372853) tremelimumab was administered intravenously to patients with mRCC at doses of 6, 10, or 15 mg/kg every 12 weeks in combination with sunitinib at 50 mg daily for 4 weeks, followed by 2 weeks or 37.5 mg daily [58]. Two of the five patients receiving tremelimumab 6 mg experienced unexpected rapid onset renal failure with sunitinib 50 mg, and one of the seven patients receiving tremelimumab 10 mg/kg plus sunitinib 37.5 mg died suddenly. The expansion cohort was treated with tremelimumab 10 mg/kg plus sunitinib 37.5 mg. However, dose-limiting toxicities were observed in three or seven patients. Of the nine patients evaluable, 43% achieved a partial response, but the regimen did not progress further due to toxicity.

Ipilimumab (anti-CTLA-4 antibody) and Nivolumab (anti-PD-1 antibody)

In a Phase 3 Checkmate 214 study in advanced RCC (NCT 02210117), 1096 patients were randomized to receive the combination of ipilimumab and nivolumab (n=550) and sunitinib (n=546). The co-primary endpoints of this study according to IMDC criteria were OS, PFS, and ORR (Objective Response Rate) in intermediate and low risk patients. ORR, PFS, and OS in the favorable risk group, and incidence of adverse events (AEs) in patients were secondary endpoints. In intermediate/low risk patients, combination therapy was associated with improved survival (HR: 0.63; 99.8% confidence interval: 0.44-0.89) and ORR (42 vs 27%), but no PFS reached the prespecified threshold (HR: 0.82; 99.1% confidence interval: 0.64-1.05). Of note, the combination of ipilimumab and nivolumab also improved the complete response rate (9 vs 1%). In patients at favorable risk, the combination did not result in a benefit in OS and ORR compared with sunitinib, however, complete response rates significantly favored the nivolumab and ipilimumab arms (11 vs 6%). There were 8 treatment-related deaths in the combination arm and 4 in the sunitinib arm. Increase in lipase, fatigue, diarrhea, rash, nausea, and decrease in appetite, and asthenia were the most common high-level AEs in the combination arm, while hypertension, palmar-plantar redness, fatigue, diarrhea, lipase increased, asthenia, vomiting, and anemia were the most common high-level AEs in the sunitinib arm. Despite this, the quality of life assessment showed that the combination arm was significantly better than the sunitinib arm [59, 60]. There was a statistically significant improvement in overall response rate (ORR) compared with sunitinib standard of care in first-line treatment of intermediate and low-risk patients [61]. However, nivolumab in combination with ipilimumab is approved as a representative first-line treatment for patients with mRCC at intermediate or low risk of IMDC [62]. Studies described in the above paragraph are summarized in **Table 1**.

Discussion

With the advent of ICI, we are entering a new era of systemic treatment for RCC. These agents are capable of restoring an immune response against tumors by inhibiting specific immune checkpoint receptors or ligands, such as programmed death receptor 1/programmed death receptor ligand 1 (PD-I/PD-L1) and cytotoxic T lymphocyte associated protein 4 (CTLA-4). More recently, the administration of immune checkpoint inhibitors has also proven to be an effective option for previously untreated patients [63]. In this case, two combination strategy have been tested: combinations between different immune checkpoint inhibitors and combinations between immune checkpoint inhibitors and targeting agents. Nivolumab monotherapy, ipilimumab plus nivolumab, and ICI plus VEGFR TKIs are now established as part of the standard of care for advanced RCC. The choice between these ICI-containing regimens remains unclear pending further long-term data. Interferon-monotherapy in combination with bevacizumab is no longer recommended in the first-line treatment of patients with Interferon-α. The immune checkpoint inhibitor nivolumab improves overall survival, 1-year mortality, adverse events, and health-related quality of life in participants with pretreated metastatic renal cell carcinoma and is an evidence-based option after failure of VEGF-targeted therapy. The combination of ipilimumab and nivolumab appears to be the first-line treatment of choice for patients with mRCC,

once available and patients are eligible for immunotherapy [64]. According to the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) [61], combination of nivolumab and ipilimumab improves survival and other clinical outcomes compared with sunitinib in patients at intermediate or low risk as the first-line treatment of mRCC patients with IMDC intermediate or low risk disease [62]. Immunotherapy has shown great promise in the treatment of many solid tumors, including RCC, non-small cell lung cancer, and melanoma, with sustained benefit, although the number of complete responses to monotherapy remains low in selected patient groups. Combination therapy appears to be the next logical approach that may improve durable survival, and there is increasing evidence to support this.

Conclusions

The first-line treatment modality for ccRCC has changed, particularly in intermediate/low risk patients, with the addition of nivolumab and ipilimumab in combination. However, more studies and better designed further studies are needed to explore the possibility and effectiveness of different drug combinations, while still focusing on their side effects, leading to better tumour outcomes. Future studies will likely identify biomarkers of subsequent immune response to better select candidates for neoadjuvant immunotherapy.

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Ethical policy

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Approval from institutional ethical committee was taken.

Availability of data and materials

All data generated or analysed during this study are included in this publication.

Author contributions

Juan Wu conceptualised, designed the study and was responsible for the writing of the original draft. Ya-fei Ren, Jun Xie and Dong-sheng Li reviewed and revised the manuscript. All authors approved the version of the manuscript to be submitted.

Competing interests

The authors declare no conflict of interest.

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