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A novel therapeutic strategy of combined CAXII inhibitors and anti–PD-1 antibodies in HCC treatment

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RESEARCH

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ABSTRACT

Hepatocellular carcinoma (HCC) is a lethal malignancy with the lack of effective treatments particularly for the disease at advanced stage. Even though immune checkpoint inhibitors (ICIs) have made a great progress in the treatment of HCC, enduring and ideal clinical benefits still cannot be achieved in a plenty of patients with HCC. Therefore, novel and refined combinations of ICIs are needed for enhancing the therapeutic effect. The latest study has reported that CAXII inhibitor (CAXIIi), as a novel type of anticancer drug, can modify immunosuppressive stress mediated by hypoxic/acidic metabolism, and regulate the expression of CCL8 by mediating the functions of monocytes and macrophages, consequently improving anti-tumor immunity and enhancing the therapeutic effect of PD-1 inhibitor in HCC, which shines a light on improving combined immunotherapy. This perspective aims to ignite enthusiasm to explore the application of CAXIIis with solid anti-tumor efficacy in combination with immunotherapy for HCC and related mechanisms.

Key Words

HCC, CAXII inhibitor, combined immunotherapy, CCL8, macrophages

Introduction

Hepatocellular carcinoma (HCC) is the most common histological type in primary liver cancer, which occurs predominantly in individuals with chronic liver disease or cirrhosis. HCC is the fourth leading cause of cancer-related death worldwide, and its incidence has been rising over the last 20 years globally[1,2]. It has been expected to keep increasing until 2030 in some countries, including the United State, and has become the fourth of the most common cancer in China. Patients with early-stage HCC can receive radical treatment, such as local ablation, surgical resection, or liver transplantation. However, a high recurrence rate still exists (five-year survival rate after surgery is only about 35%)[3,4]. In addition, over a half of HCC patients have already been at advanced stage when diagnosed due to the lack of sensitive and specific diagnostic tools in clinic, and the suitable treatment strategies and corresponding curative efficacy are extremely limited.

Since most HCCs are derived from chronic inflammatory liver damage (e.g., hepatitis B virus-related), such a disease was considered as inflammation-induced related cancer. Therefore, HCC patients are theoretically considered to benefit from immunotherapy[5]. Various combination therapy strategies based on immune checkpoint inhibitors (ICIs) have brought new opportunities for the treatment of HCC. however, a considerable number of HCC patients are still unable to gain enduring and ideal clinical benefits. Therefore, how to explore new ICIs combined drug strategies to retrieve better objective response rate, has become a hot and difficult issue in the current international frontier[6-8].

Current status of CAXII inhibitor application in cancer treatment

Carbonic anhydrase XII (CAXII) is a transmembrane zinc metalloenzyme involved in the regulation of the tumor microenvironment, contributing to tumor cell proliferation, invasion, migration, and their pluripotency[9]. It has been reported that CAXII is over-expressed in HCC, and its level is significantly negatively correlated with the prognosis of HCC patients, which may act as an independent prognostic factor[10].

A highly hypoxic tumor microenvironment is a hallmark for human cancer including HCC, due to its rapid growth rate and surrounding fibrotic tissue produced by persistent chronic inflammation. The overproduction of pyruvate, lactate, and carbonic acid in response to hypoxia aggravates the hypoxic/acidic microenvironment, leading to the enhancement of tumor invasion, tumor immune surveillance escape and local inflammation. As a regulator of hypoxic stress and acidity, CAXII can affect the tumor microenvironment by regulating proteins such as 14V-ATPase and 15V-ATPase, thereby promoting HCC progression. Therefore, CAXII inhibitor is thought to be a novel anti-HCC agent, which can control HCC progression and reduce the immunosuppressive stress *via* the regulation of hypoxic/acidic metabolism. However, the investigation of the anti-tumor effects and mechanisms of CAXII inhibitors is still in fragmentation. The development and exploration of anti-tumor CAXII inhibitors has become a new global research hotspot[11,12].

CAXII inhibitor on cancer treatment has just started recently. The first highly selective small molecular inhibitor of both CAIX/CAXII (SLC-0111) had completed its phase 1 clinical trial with promising results. Briefly, 17 patients with 10 different cancer types including 1 HCC patients were recruited on the inhibitor in this study. The results showed that the inhibitor was safe in patients with previously treated advanced solid tumors[13]. Moreover, a multi-center, open-label Phase 1b study of SLC-0111 in combination with Gemcitabine for Metastatic Pancreatic Ductal Cancer in Subjects Positive for CAIX has also started since 2018. Nevertheless, to our knowledge, no other attempt has been made to investigate the therapeutic effect of CAXII inhibitor in HCC treatment so far, which is still a desert-like field.

Macrophage is effective target for sensitizing immunotherapy

Macrophages, as one of the most abundant immune cells in tissues, are highly heterogeneous and can switch between different functions in the context of its niches where they are located. They function to either kill tumor cells or promote tumor progression. Abundant M2 macrophages were positively associated with poor survival in patients with breast cancer[14]. Recent studies have found that effectively interfering with macrophages is a potential strategy to treat cancer.

A study recently published in Nature Immunology has reported that the metabolic reprogramming of macrophages can eventually inhibits tumor growth by regulating T cells[15-17]. Briefly, with tumors developing, macrophages interact with the cancer cells to produce a response protein ("protein kinase R" (PKR)-like endoplasmic reticulum kinase, PERK), which is involved in the remodeling of several key pathways of metabolism in macrophages. It has been found that blocking the expression of PERK can inhibit the downstream metabolic signaling in tumor infiltrated macrophages, resulting in more effector T cells to fight the cancer cells, and enhancing the efficacy of PD-1 inhibitor[18]. Therefore, targeting or editing metabolism of macrophages has been thought to be a novel therapeutic treatment in combination with PD-1 inhibitor. Furthermore, it has been reported recently that CAXII inhibitor can interfere the metabolism of macrophages, and promote the therapeutic effect of PD-1 inhibitor in HCC treatment[19].

CAXII inhibitor can regulate macrophages

Evidence has shown that CAXII was the most significantly up-regulated gene in tumor-infiltrating monocytes compared to monocytes in non-tumor liver tissue, among all αCA family genes, by comparing differentially expressed genes in monocytes purified from HCC patient tumors and paired non-tumor liver tissues[18]. Moreover, the increased expression level of CAXII mRNA in tumor-infiltrating monocytes but not in other CD14+ cell components in both tumor tissue and non-tumor liver tissue indicates that CAXII may contribute to HCC progression. It was also shown a positive correlation between the expression level of CAXII glucose transporter GLUT1 in in tumor-purified CD14+ cells, which may participate in the glycolytic switch in tumor-infiltrating monocytes and macrophages[16].

In addition, glycolysis inhibitor 2-deoxyglucose (2-DG) or PFKFB3 (a key glycolytic enzyme) inhibitor can effectively reduce the expression of CAXII mRNA and protein levels in HepG2 TSN-treated monocytes (peripheral blood purified CD14+ cells from healthy subjects). Meanwhile, tumor-triggered glycolytic switch in monocytes has been found to induce activation of HIF1α and production of TNF-α, IL-10, and IL-1β, which in turn synergistically upregulates CAXII expression in monocytes. Therefore, it has been thought that the glycolysis can induce CAXII upregulation through HIF1α and autocrine cytokine-dependent pathways in

monocytes and macrophages, and CAXII was also found to mediate the survival of macrophages and monocytes in an acidic microenvironment in HepG2[18].

As CCL8 (C-C motif chemokine ligand 8), a member of the CC chemotactic protein family, can recruit monocytes, T cells, eosinophils, basophils, NK cells, and dendritic cells by binding to CCR1, CCR2, CCR3, and CCR5 receptors. It acts an important immunoregulatory role in inflammatory response, antitumor immunity and acute graft-*versus*-host disease (aGVHD)[20]. Evidence has shown that the levels of MMP9, VEGFA and CCL8 were all increased in tumor monocytes, with CCL8 showing the most pronounced upregulation, compared to non-tumor monocytes. Therefore, it has been considered that glycolysis-induced upregulation of CAXII expression may be closely related to CCL8 production by tumor-associated monocytes and macrophages[22]. Moreover, CCL8, as a CC chemokine that utilizes multiple cellular receptors to attract and activate human leukocytes, was reported to significantly promote the migration of HepG2 cells, and increase the expression levels of vimentin (VIM) and SNAI1. The mRNA level of CCL8 in tumor-infiltrating monocytes was also found to be positively correlated with VIM, negatively with CDH1, and positively with the metastatic potential of HCC patients. Therefore, the CAXII/CCL8 axis is considered as a potential carcinogenic mechanism and an effective therapeutic target[20].

Further evidence displayed that, CAXII inhibitor significantly increased the therapeutic effects (including suppressing tumour growth, attenuating tumour metastasis and enhancing OS of mice) of anti–PD-1 antibody on HCC compared to either single CAXII inhibitor group or single anti–PD-1 antibody group ($P<0.05$) *in vivo*, respectively. In addition, CAXII inhibitor has also been found to increase the apoptosis of macrophages, reduce their ratio in total CD45+ cells and increase the ratio of CD8+ T cells in total tumor lymphocytes[19]. Such result is partly consistent with our previous study which is targeting CAXII can effectively inhibit the development of liver cancer[14].

Selection of CAXII inhibitor for combination immunotherapy

Since the immunoregulation ability of CAXII inhibitor has been revealed, how to choose an appropriate CAXII inhibitor synergizing PD-1 mAb treatment has become a crucial problem. In addition, some CAXII inhibitors CAXIIisha ve been found to exert excellent anti-cancer effects on cancer cells by monotherapy[11-13]. Therefore, it would be more intriguing if the tumor-suppressive effect could be further enhanced by combining with PD-1 inhibitors. Based on what we found in our previous study, Tiliroside (TS) is presented as a potential candidate.

Tribulus terrestris L. (TT), a plant, can be found in many regions of Asia and Africa, and has been used in Traditional Chinese Medicine and Ayurvedic medicine as an herb to treat liver diseases for thousands of years. TS is extracted from the herb, and is one of the most important components of TT[22]. TS possesses anti-inflammatory, anticholinesterase and antioxidant activities. Moreover, our recent study further revealed its multiple anti-cancer effects on HCC cells, as a typical CAXIIi. Such an agent can inhibit the proliferation, colony formation, migration, 3D organoid formation and invasive abilities by regulating apoptosis, stemness in HCC cells, but with a low toxicity to liver normal cells[11]. Therefore, TS seems to be a promising candidate in combination with PD-1 inhibitor to improve the immunotherapy efficacy Figure 1.

Conclusion

Accumulating evidence demonstrated the anti-cancer effect of CAXII inhibitors in different types of cancers, including HCC. Therefore, such agents are considered to be a promising novel anti-HCC drugs that can suppress HCC progression. Moreover, CAXII inhibitor has been found to reduce the immunosuppressive stress mediated by hypoxic/acidic metabolism, regulate the expression of CCL8 by mediating the functions of monocytes and macrophages, therefore improves anti-tumor immunity and enhances the therapeutic effect of PD-1 inhibitor in HCC. In one word, CAXII inhibitor is not only a single anti-cancer agent, but a potential sensitizer of PD-1 inhibitor. Thus, Tiliroside, as a novel CAXII inhibitor with high-efficiency and low-toxicity anticancer effect, has a potential in HCC treatment, especially in immune combination therapy. Certainly, more promising candidates of CAXIIi should also be included in future studies to explore more effective therapeutic strategies and novel therapeutic targets for HCC.

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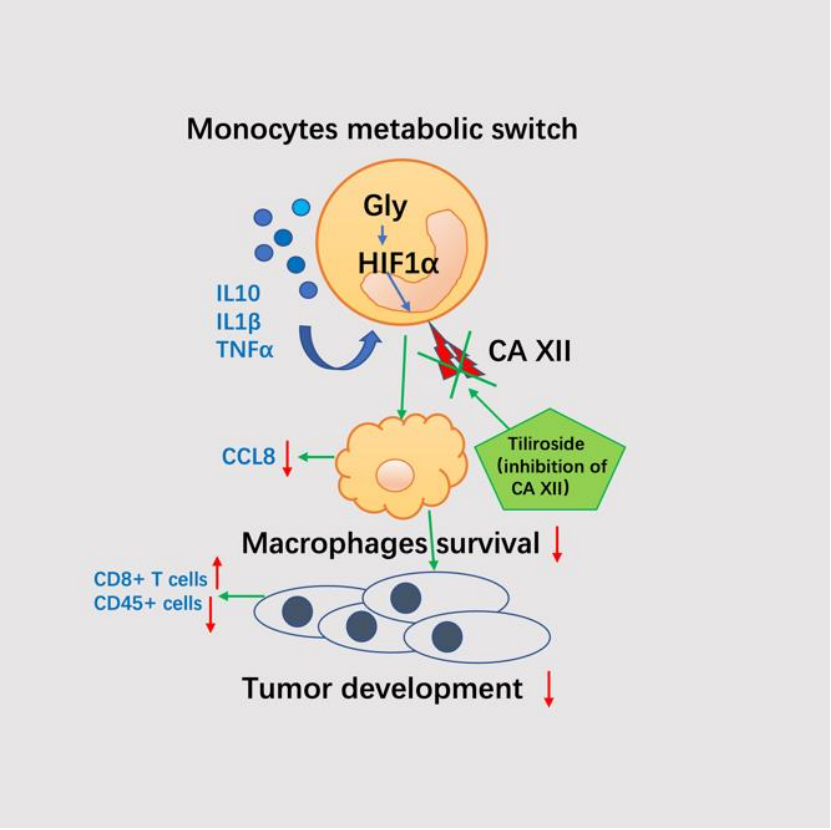
Figures

Figure1: Potential mechanism of *Tiliroside* enhancing the therapeutic effect of PD-1 inhibitors



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