REVIEW

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Potential of natural products and gut microbiome in tumor immunotherapy

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Abstract

Immunotherapy is a novel treatment approach for malignant tumors, which has opened a new journey of anti-tumor therapy. Although some patients will show a positive response to immunotherapy, unfortunately, most patients and cancer types do not achieve an ideal response to immunotherapy. Therefore, it is urgent to search for the pathogenesis of sensitized immunotherapy. This review indicates that *Fusobacterium nucleatum*, *Coprobacillus cateniformis*, *Akkermansia muciniphila*, *Bifidobacterium*, among others, as well as intestinal microbial metabolites are closely associated with resistance to anti-tumor immunotherapy. While natural products of *pectin, inulin, jujube, anthocyanins, ginseng polysaccharides, diosgenin, camu-camu,* and *Inonotus hispidus (Bull).Fr. P. Karst, Icariside I, Safflower yellow, Ganoderma lucidum,* and *Ginsenoside Rk3*, and other Chinese native medicinal compound prescriptions to boost their efficacy of anti-tumor immunotherapy through the regulation of microbiota and microbiota metabolites. However, current research mainly focuses on intestinal, liver, and lung cancer. In the future, natural products could be a viable option for treating malignant tumors, such as pancreatic, esophageal, and gastric malignancies, via sensitizing immunotherapy. Besides, the application characteristics of different types, sources and efficacy of natural products in different immune resistance scenarios also need to be further clarified through the development of future immunotherapy-related studies.

Keywords Cancer, Immunotherapy, Resistance, Gut microbiota, Nature product

Introduction

In the 1890s, American surgeon William Coley used Coley toxin to treat tumors, pioneering tumor immunotherapy. Following the use of chemotherapy, targeted therapy, and anti-angiogenesis therapy, the introduction of immunotherapy in tumor therapy is a noteworthy development. The efficacy and durability of this novel family of immune checkpoint inhibitors was impressively demonstrated. It consists of cytotoxic T-lymphocyte antigen-4 (CTLA-4) [1], programmed death ligand 1 (PD-L1), and programmed death receptor 1 inhibitors (PD-1)

[2]. An increasingly significant advancement with the management for cancer is the inclusion of solid tumors in immune checkpoint inhibitor regimens [3–6]. Immunotherapy for cancer has helped a great deal of patients, however not everyone responds the same to immune checkpoint inhibitor (ICI) treatment. Presently, antibodies against PD-1 and PD-L1 have been approved for treatment in a number of different cancer types [7–9]; however, the objective response rates to these inhibitors vary according to the type of tumor, ranging from 15 to 87% [10, 11]. Even while ICI-based immunotherapy has shown to be highly effective in improving patient outcomes for a variety of cancer types, a sizable portion of individuals fail to achieve a long-lasting response. Specifically, even among patients who respond to therapy with anti-PD-1 the best, 60–70% of patients with melanoma are unable to objectively respond to treatment. Eventually [12, 13], 20–30% of patients with tumors will experience

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recurrence or progression. To provide immunotherapy's benefits to a wider range of patients, it is imperative that methods for boosting immunotherapy's response rate and conquering immunological resistance be looked into immediately.

The gut is home to a variety of microbes that can cause illness and impact health. They change several physiological systems, such as immune function, metabolism, and digesting capacity, which has a major effect on human wellness and illness [14–18]. The importance of gut microorganisms in controlling tumor immunity has been demonstrated. They can fight immune resistance and non-response by influencing the efficacy of cancer immunotherapy and modulating anti-tumor immune responses. Consequently, regulating intestinal microorganisms offers new insights for improving the effectiveness of tumor immunotherapy and overcoming immune resistance [19–23].

Natural products are naturally occurring small molecules that have shown great potential in treating a variety of diseases [24–26]. In addition to being the main target of drug development, screening, and research, they are also essential for controlling the immune response. Many natural products have recently been discovered to have potent anti-cancer qualities. They can regulate the immune response to tumors by influencing intestinal microorganisms and enhancing the efficacy of tumor immunotherapy. Many compounds that are derived from natural products or their derivatives are either being evaluated in clinical studies or are being used as cancer therapies. This review aims to explain what links intestinal microbiota and the immune response to malignancies, as well as natural chemicals that alter intestinal microorganisms to improve the immune response to immunotherapy. This will further the development of tumor immunotherapy and enhance clinical intervention strategies for immune resistance.

The connection among tumor immune resistance and gut microbiota

The variety of microbes and their metabolites that live in the intestines are known to as "gut microbiota". These microbes influence the body's medicine and diet, among other things. Additionally, they contribute to the modification of immunological state [14, 16, 17]. There are currently research that support the link among gut flora and anti-tumor immune therapy efficacy [19–21]. For example, a group at Peking University Cancer Hospital discovered that the ratio of Prevosia/Bacteroides in patients positively correlated with the effectiveness in anti-PD-1/PD-L1 immune therapy. They also discovered that the abundance of *Lachnospiraceae*, *Prevotella*, and *Ruminococcaceae* was significantly higher in some response subgroups. The analysis was done using 16S rRNA sequencing of stool samples [27]. Furthermore, individuals with varying levels of treatment efficiency showed differences in pathways linked to the manufacture of nucleosides and nucleotides, lipids, glucose metabolism, and short chain fatty acids (SCFAs), showing the possible impact to the microbiome of the gut for the therapeutic effect of immunotherapy. Connection among tumor immune resistance and gut microbiota is shown in Fig. 1.

Relentlessly mounting data from experimental and clinical trials demonstrates the beneficial contribution in the intestinal microbiota and its metabolites to immunotherapy response, being a major modulator of immunity against tumors during immunotherapy, mediated by a "gut microbiota-metabolism-immune interaction" pathway [15, 28]. Changing the gut microbiome offers fresh perspectives on enhancing the immune system's capacity to fight cancer. This section centers on the connection between DRTI and gut microbes, which could offer fresh perspectives on immunotherapy resistance.

Gut microbiota
Fusobacterium nucleatum (F.nucleatum)
For CRC, *F. nucleatum* is the most significant pathogen. For the first time, it was discovered in a study that *F. nucleatum* and its metabolite succinic acid exhibited drug resistance when used in CRC immunotherapy [29]. Researchers discovered that patients with advanced colorectal cancer (CRC) who were unresponsive to immunotherapy had higher serum succinic acid levels and more *F. nucleatum* in their feces, both of which were linked to a poor prognosis. *F.nucleatum* inhibits the cGAS-IFN-β pathway, decreases the production of Th1 chemokines CCL5 and CXCL10, and downregulates the chemotactic and activating function of CD8$^+$ T cells by secreting the metabolite succinic acid, which stimulates the SUNCR1-HIF-1α-EZH2 axis of the succinic acid receptor in tumor cells. As a result, PD-1 monoclonal antibody's effectiveness in CRC might be diminished. According to in *vivo* research, the antibiotic metronidazole may reduce a certain amount about *F. nucleatum* at the colon and the serum level of succinic acid, thereby re-sensitizing CRC to immunotherapy.

Coprobacillus cateniformis
While PD-1 is a common docking partner for both PD-L1 and PD-L2, PD-L2 can additionally connect to RGMb, which is a binding receptor for PD-L2. The findings showed that resistance to PD-1 or PD-L1 inhibitors caused via the microbiota can be overcome by inhibiting the PD-L2-RGMb interaction [30]. When mice were reconstituted with healthy human microbiota (HMB) instead of germ-free and antibiotic-treated mice injected
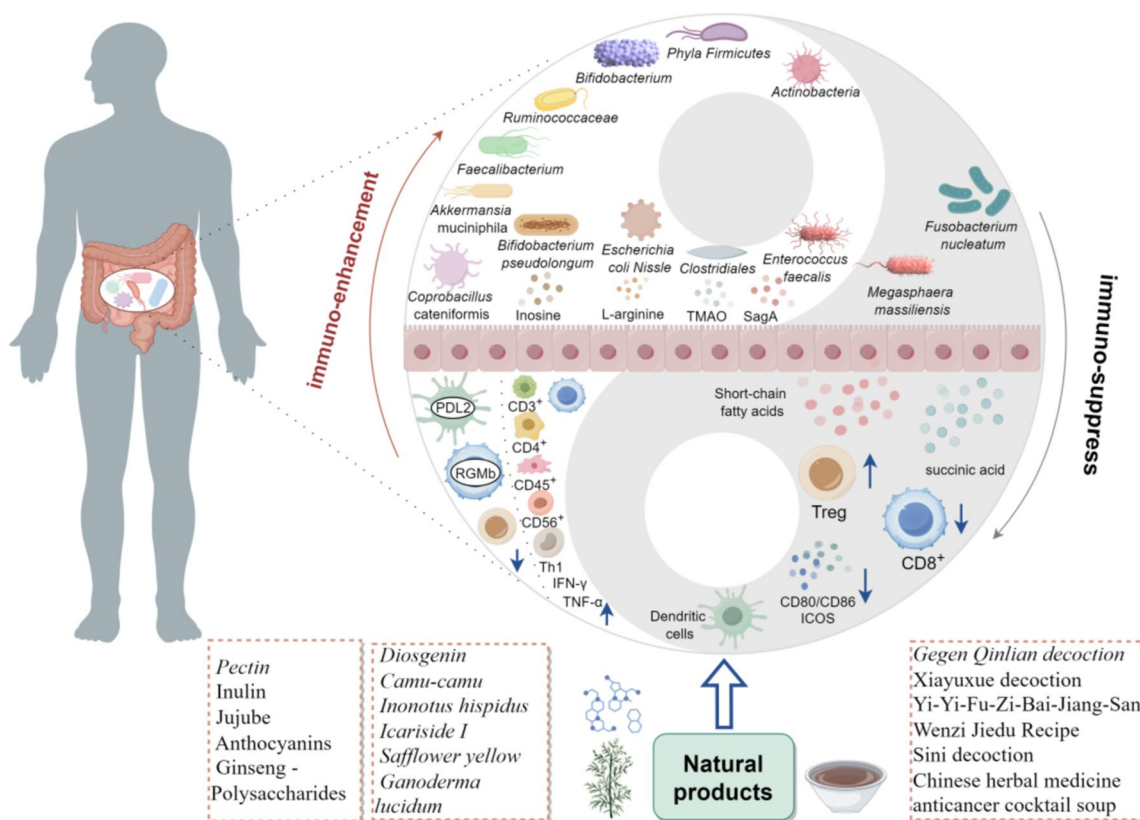
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Fig. 1 The relationship and mechanism between gut microbiome and tumor immune resistance

with MC38 colon cancer, the efficacy for anti-PD-1 / PD-L1 immunotherapy against tumors was increased. By decreasing the expression of RGMb in CD8+ T cells and PD-L2 in dendritic cells, microbiota can strengthen immunity against tumors. Conversely, the reduction of PD-L2 expression is required for the symbiotic gut microbiota *Coprobacillus cateniformis* to have an effect on tumor fighting immunity. Anti-tumor immunity can be strengthened when anti-RGMB and anti-PD-L1 therapy are combined.

Akkermansia muciniphila

Antibiotic therapy (ATB) is a major contributor to gut microbiota imbalance and has the potential to reduce PD-1's therapeutic efficacy and develop PD-1 resistance. It was discovered that individuals who progression-free survival (PFS) longer than six months have greater metagenomic species and gut microbial gene abundance [31]. The majority of *kkermansia muciniphila* (*A. muciniphila*) is found in patients with stable disease (SD) and partial response (PR) who are receiving effective PD-1 medication therapy. *Firmicutes* is most abundant in long-lived patients. Additionally, *Enterococcus hirae* (*E. hirae*) was most frequently discovered in the stool of

PR patients, supporting the association with intestinal microbiota and the effectiveness with immune therapy. *A. muciniphila* bacteria were also found in 69% of PR patients and 58% of SD stool samples. *A. muciniphila* or *A. muciniphila* & *E.hirae* administered intragastrically to mice reversed their PD-1 response to antibiotic therapy. Another study [32] conducted elsewhere discovered that patients via metastatic renal cell carcinoma (RCC) had higher relative abundances and prevalences of *A. muciniphila* and *B. salyersiae* in their stool among ICB responders (Rs) compared to non-responders (NRs). NR-fecal microbiota oral transplantation (FMT) (without *A. muciniphil* or *B.salyersiae*) with the immunostimulant *A. muciniphila* or *B. salyersiae* or R-FMT can restore sensitivity to immunotherapy prior to every ICB period. Because the gut microbiota regulates immunity against tumors in cancer patients, it may be possible to eradicate or reverse patients' immunotherapy resistance by modifying the gut ecosystem.

Bifidobacterium

Researchers led by Professor Thomas F. Gajewski of the University of Chicago examined certain bacteria in 42 patients about advanced melanoma both before and after

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receiving anti-PD-1 therapy. They found that the amounts of *Bifidobacterium longum*, *Collinsella aero faciens*, and *Enterococcus faecium* were higher in individuals who reacted favorably to immunotherapy [33]. Among them, *Bifidobacterium* OTU (559527) had the highest Spearman correlation with *Bifidobacterium* longum, and mice receiving these bacteria responded more strongly to T cells and immunotherapy. Moreover [34], *Bifidobacterium* was linked favorably to anti-tumor T cell responses. Mice given with *Bifidobacterium* had considerably better tumor control than mice not treated with *Bifidobacterium*, and this improvement was supported by an effective induction of peripheral tumor-specific T cells and an increase in the buildup of CD8+ T cells specific to the antigen within tumors. As a result, oral *Bifidobacterium* treatment could enhance the effectiveness of ICIs, increase anti-tumor immunity, and maybe strengthen the therapeutic effect of immune checkpoint inhibitors such as CD47, PD-1/PDL-1, and CTLA4 inhibitors.

Ruminococcaceae
Researchers led by Professor J.A. Wilgo of the MD Anderson Cancer Center have discovered [35] that Rs to melanoma PD-1 immunotherapy possess substantially greater alpha diversity and proportion of the *Ruminococcaceae* family, and that R has more functional anabolic pathways of gut bacteria than NRs. There is also increased anti-tumor and systemic immunity. Individuals possessing a "good" gut microbiome, defined as having a high variety and abundance of *Rumenococcaceae/Faecalis*, were found to mediate a strengthened systemically and anti-tumor immune system response. This was achieved by improved activity of effector T cells in the tumor microenvironment and higher antigen presentation. Due to restricted lymphocyte and myeloid infiltration, decreased antigen-presenting potential within the tumor, and poor variety and high relative abundance of *Bacteroides* in their gut microbiome, those who had "unfavorable" gut microbiomes mediated a compromised systemic and anti-tumor immune response. Furthermore, it was discovered that patients with high gut microbial abundances of *Clostridiales*, *Ruminococcaceae*, or *Faecalibacterium* showed increased systemic circulation levels of effector CD4+ and CD8+ T cells together with an anti-PD-1 therapy response. Conversely, patients whose gut microbiome included a high concentration of *Bacteroidales* had higher amounts of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) in the bloodstream, along with a lower cytokine response.

Faecalibacterium
Using the method of 16S rRNA gene sequencing, a study assessed the diversity of the microbes in the feces at baseline and prior to each treatment in patients of metastatic melanoma (MM) receiving ipilimumab [36], an immune checkpoint inhibitor that targets CTLA-4. Contrary to cluster B patients, that baseline microbiota had been altered primarily Bacteroides, it was discovered that the baseline microbiota for cluster A was enriched for *Faecalibacterium* along with other *Firmicutes* as unclassified *Ruminococcaceae*, and *Clostridium* XIVa. *Blautia* and *Clostridium* XIVa exhibited prolonged OS and PFS. Cluster A and long-term clinical benefit were linked to a low number of peripheral blood tregs and a considerably reduced percentage of baseline α4β7 CD4+ and α4β7 CD8+ T cells. It is clear that the clinical advantage of increased efficacy to immunotherapy targeting CTLA-4 is connected with a baseline gut microbiota richer in *Faecalibacterium* and other *Firmicutes*. There is more *Bacteroidetes*, especially the *Bacteroidetes* genus, among individuals that fail to respond well with immunological treatment.

Phyla Firmicutes and Actinobacteria
In the single-arm clinical trial NCT03341143 [37], it was discovered that following a single FMT in PD-1 inhibitor Rs patients and non-responders (NRs), the flora of complete response (CR) donors in PD-1 inhibitor Rs patients showed higher α-diversity. Individuals with PD-1 refractory melanoma are those who have not responded to PD-1 inhibitors in the past, either on their own or in combination with CTLA-4 or the experimental medication. While the majority of the groups with a substantial drop in Rs belonged to the phylum *Bacteroidetes*, the majority of the bacteria that were considerably increased in Rs related to the phyla *Firmicutes* (families *Lachnospiraceae* and *Ruminococcaceae*) and *Actinobacteria* (families *Bifidobacteriaceae* and *Coriobacteriaceae*). In contrast to NRs, Rs exhibited enhanced stimulation of CD8+ T and MAIT cells, a higher percentage of CD56+CD8+ T cells following treatment, and a lower frequency of circulating IL-8 and IL-8-producing myeloid cells. We conclude that FMT combined with PD-1 blockers may change the tumor microenvironment and boost sensitivity to PD-1 inhibitors for the positive effects of immunotherapy in some patients experiencing advanced melanoma.

Gut microbiota metabolites
There are intricate relationships between gut microorganisms, microbial metabolites, and host cells. The gut microbiome influences several energy metabolism systems in *vivo*. By controlling the immune system, inflammatory response, TME signaling pathway, causing epigenetic alteration, and regulating the inflammatory response, the metabolites generated from gut bacteria play a variety of roles in carcinogenesis and development.

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They've got an essential contribution to the immune response against tumors.

Peptidoglycan hydrolase secretes antigen A

Peptidoglycan hydrolase secretes antigen A (SagA), an enzyme secreted by *Enterococcus* that breaks down bacterial cell wall components and releases fragments of muramyl peptide. These peptide fragments then function as signaling molecules to stimulate the innate immune sensor protein NOD2 and improve the response to immunotherapy. Studies have found [38] that specific *Enterococcus*, mainly *Enterococcus faecium*, *E.durans*, *E.irae* and *E.Meundtii*, have the ability to enhance the potency of anti-PD-L1 immunotherapy for cancer. By comparing the four types of *Enterococcus* that can support immunotherapy and the ineffective *Enterococcus faecalis*, SagA is found in the active *Enterococcus faecium*, and if the *Enterococcus faecalis* has SagA through transgenic technology, they also have the ability to support immunotherapy. If the non-*enterococcus* family of *Lactococcus lactis* gets SagA, it can also become a small anti-cancer helper. Furthermore, following *Enterococcus faecalis* colonization, there was an increase in the overall quantity of CD45$^+$ leukocytes and CD3$^+$ lymphocytes within the tumor, as well as an increase in the proportion of CD8$^+$ T cells infiltrating the tumor. These results imply that anti-tumor cytotoxic T cells boosted innate immunity, created an environment that was favorable for immunotherapy, and improved the effectiveness of immunotherapy.

Trimethylamine N-oxide

Initially, it was suggested that trimethylamine N-oxide (TMAO) played a part in preserving the functional and structural strength of proteins. The theory that TMAO can cause inflammation and immunological activation is supported by recent research. Tumors with an active immunological microcosm were shown to have higher levels of the *Clostridiales*-related metabolite TMAO [39]. The combination intramuscular administration of TMAO has a more substantial suppressive impact on growth of tumors than anti-PD-1 antibody alone. It improves immunotherapy response. Through the activation of the endoplasmic reticulum kinase PERK, TMAO causes tumor cells to undergo pyroptosis. In *vivo* triple-negative breast cancer (TNBC) is improved by this procedure in terms of CD8$^+$ T cell-mediated immunity against tumors. It stimulates M1 macrophages and CD8$^+$ T cell infiltration, which eventually improves CD8$^+$ T cell activity, and increases interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) levels. According to a different study [40], TMAO directly boosts effector T cells' ability to fight tumors, boosts macrophage immune response,

and lowers the incidence of pancreatic ductal adenocarcinoma (PDAC). Increased survival of patients and an anti-PD-1 response were substantially positively correlated with levels of CUTC$^+$ bacteria, a crucial enzyme that catalyzes the synthesis of TMAO. As a result, the gut microbiota's generation of TMAO stimulates an advantageous reaction to immunotherapy.

Short-chain fatty acids

Short-chain fatty acids (SCFAs) are vital substances produced by the gut microbiota from fermented fiber in the diet. The amount of SCFAs in the lumen of the intestinal tract increases when complex, indigestible carbohydrates are consumed. *Megasphaera massiliensis*, a low-abundance human gut bacterium, may produce substantial amounts of valerate SCFAs and butyric acid. Elevated levels in the blood of the compound butyrate and propionate have been linked to increased proportion of treg cells and resistance to CTLA-4 inhibition [41]. Butyric acid was discovered to prevent cancer-specific T cells and memory T cells from accumulating in *vivo*, as well as the increased levels of CD80/CD86 on anti-CTLA-4-induced dendritic cells and ICOS in T cells. SCFA may have restricted the anti-CTLA-4 response in patients where moderate iprilizumab with elevated blood butyrate concentrations produced memory, buildup of ICOS$^+$CD4$^+$ T cells, and infiltration of IL-2. Furthermore [42], valerate or supernatant was used to treat CAR T cells and cytotoxic T lymphocytes (CTLs) that were co-cultured with *Megasphaera massiliensis*. It has the ability to inhibit class I histone deacetylases (HDACs), increase the activity of mTOR, and improve the ability to combat tumors of CTL and CAR-T cells. This is a great illustration of how microbial metabolites might be used therapeutically to improve immunotherapy by affecting both metabolism and epigenetic condition of CTL reprogramming.

Inosine

The main source of isosine, a purine metabolite, is the symbiotic bacterium *Bifidobacterium pseudolongum* in the intestinal lumen. It was discovered [42] that inosine can stimulate mucosal Th1 cell proliferation, allowing the cells to move to the tumor microenvironment and strengthen the tumor-opposing immune response. Furthermore, inosine activates A2RR-CAMP-PKA-dependent signaling, that stimulates Th1 proliferation as well as amplifies the immune response against tumors during immune checkpoint blockage (ICB). In addition, by increasing T-cell-mediated cytotoxicity, inosine can produce a "inflammatory" immunological milieu [43]. This has the potential to make ICB effective against cancers such as melanoma and breast cancer, which are usually

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resistant to it. Additionally, inosine can boost anti-PD-1 and anti-CTLA-4 antibody efficacy.

Succinic acid

Succinic acid produced from *F. ucleanum* suppresses the anti-tumor response by preventing CD8^{+} T lymphocytes from entering the tumor microenvironment (TME) in *vivo* and blocking the cGAS interferon-β pathway. Reducing intestinal *F. nucleanum* abundance, which lowers serum succinic acid stages, and raising succinic acid levels in individuals at advanced CRC who do not react to immunotherapy can resensitize tumors through in *vivo* immunotherapy [29].

L-arginine

L-arginine has been recognized in the past as a nutrient and has recently been identified as an important immune stimulant. The surface of most tumor cells overexpresses arginine-degrading enzymes, leading to a scarcity of L-arginine in the tumor environment. Apoptosis and T cell malfunction are the outcomes of this paucity [44]. Moreover, L-arginine degradation lowers CD3 expression and decreases T cell responsiveness. Combining treatment with α-PD-L1 antibody and L-arginine was reported to increase the survival rate of patients with osteosarcoma [45]. Additionally, it raises the percentage of CD8^{+} T cells as well as CD86^{+}CD11c^{+} dendritic cells within the spleen in *vivo*, upregulates serum IFN-γ levels, and increases the quantity and function of CD8^{+} T cells in tumor-draining lymph nodes. Supplementing with L-arginine boosted the reaction of PD-L1 blocking antibodies and raised the quantity of tumor-infiltrating lymphocytes (TILs). In one study [46], an engineered probiotic bacterium of *Escherichia coli Nissle* 1917 was created using synthetic biology and modified microbial therapeutics. This strain colonizes tumors and produces L-arginine from accumulated waste ammonia. When paired with PD-L1 blocking antibodies, just one boost in L-arginine content within the tumor may increase the amount of T cells invading the tumor and strengthen the immune response.

Natural products modulate DRTI through microbiota and microbiota metabolites

The clinical response rates to immune checkpoint inhibitors (anti-PD-1/PD-L1) range restricted 10–30% [47], mostly because of the complex tumor micro environment and immune reconstitution mechanisms employed by cancer cells [48]. Modification of the gut microbiota has been frequently applied to control cancer immunotherapy [49].

A growing body of research indicates that a variety of natural compounds, including *curcumin*, *ginseng*, and *berberine*, may improve immune function by changing the tight connection structure, microbial metabolites, and gut microbiota composition. All things considered, elucidating the immunoregulatory impacts of various natural products on gut microbiota may result in novel developments in immunotherapy and cancer treatment. The molecular formula of natural products with DRTI is detailed in Fig. 2.

Natural active ingredients

Pectin

Pectin is a commonly ingested soluble fiber that has been shown to reduce gut microbiota imbalances and inhibit CRC by preventing tumor cells from proliferating [50, 51]. Zhang et al. [51] discovered that pectin may improve the anti-PD-1 effectiveness and govern the gut microbiota in mice with humanized microbiomes. Pectin supplementation also significantly increased the levels of several probiotics, including *Erysipelotrichaceae*, *Bifidobacteriaceae*, *Lactobacilllaceae*, and *Ruminococcaceae*, and it can stimulate the infiltration of T cells into the TME and elicit an anti-tumor impact that is dependent on CD8^{+} T cells. Moreover, *Pectin* can help correct an imbalance of the microbes in the gut. The relative number of microorganisms that produce butyrate increased dramatically within the anti-PD-1 combined *Pectin* group, indicating that butyrate plays a positive role in anti-PD-1 monoclonal antibody.

Inulin

Dietary fiber known as *inulin*, which is widely distributed in the *Asteraceae* family [52], has been described to be a prebiotic that stimulates the proliferation of *Bifidobacterium* and *Lactobacilli* and strengthens the body's defenses against anti-PD-1 [53, 54]. The proportions of important commensal microorganisms, including *Lactobacillus*, *Roseburia*, and *Akkermansia*—which have been identified as the main producers of SCFAs like acetate, propionate, and butyrate—were elevated by oral delivery of *inulin*-gel therapies [55, 56]. The systemic anti-tumor efficacy of α-PD-1 treatment can be increased by it as it can promote memory recall responses for interferon-γ^{+} CD8^{+} T cells with the development of stem-like T-cell factor-1^{+} PD-1^{+} CD8^{+} T cells within the TME. Furthermore, the concentrations of hippurate, 1-methyl-L-histine, 3-methyl-L-histine, L-arginine, and oleate were significantly elevated by *inulin* gel with α-PD-1 therapy. It's noteworthy to consider that those metabolites have been scientifically linked to a high immunotherapy response rate (AUC=0.91) [57]. Therefore, systemically memory T cell responses, gut microbiota modulation, and improved responsiveness to checkpoint inhibitors can all be achieved using oral *inulin* gels.
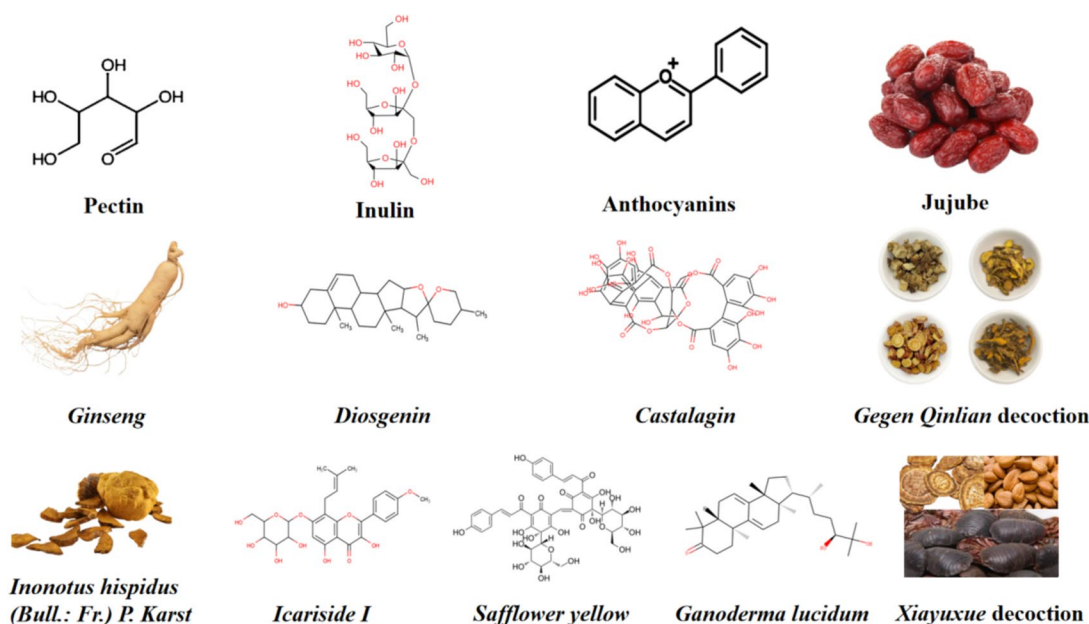
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Fig. 2 Molecular formula of natural products with DRTI

Jujube

The natural fruit known technically as *Ziziphus jujuba Mill.*, or *jujube* (Chinese date) is frequently utilized in traditional Chinese medicine(TCM). Its antibacterial capabilities, anticancer properties, immunostimulating traits, and antioxidant as well as anti-inflammatory actions are only a few of its many medical benefits [58–62]. According to a study, αPD-L1's increased antitumor efficiency can be attributed to the use of ultrafine *Jujube* powder (JP) [63]. The substantial amount of *Clostridiales*, such as *Lactobacillaceae*, and *Ruminococcaceae*, along with the augmentation of SCFA production—which has a positive correlation to CD8+ T-cell infiltration in the tumor—indicate that JP modifies the composition of the gut microbiota. JP of fewer particles can boost the number of CD8+ T cells in lymphocytes that infiltrate tumors [64, 65]. As a result, the response of patients to anti-PD-L1 therapy for CRC was significantly enhanced. It demonstrated that ultrafine JP can enhance systemic immunity and tumor immune infiltration by changing the gut microbiota's composition, which enhances αPD-L1's antitumor response.

Anthocyanins

Polyphenolic chemicals called *Anthocyanins* have been shown to have antioxidant properties and to have antitumor properties against colon and breast malignancies [66, 67]. Furthermore, *Anthocyanins* metabolic products generated by gut microbes may be responsible for the anticancer effects [68]. It was established by a study that supplementing with oral *Bilberry anthocyanin* (BA) extracts improves αPD-L1's anti-tumor activity by changing the gut microbiota's makeup [69]. The quantity of *Lactobacillus johnsonii* and *Clostridia* may rise in response to BA extracts. *Clostridia* overexpression would encourage SCFA synthesis and trigger T cell reactions [70, 71]. Furthermore, butyrate concentration and percentage in stools can both be increased by BA, and intratumoral CD8+ T cell infiltrations can be strengthened [72]. One of *Lactobacillus Johnsonii's* key functions is immunoregulation. Although oral supplementation of BA extracts with αPD-L1 increased their anti-tumor efficacy, administering BA extracts alone had no discernible effect on the rate at which tumors grow. When combined, BA may affect the gut microbiota's composition, ICIs' efficacy is thereby increased.

Ginseng polysaccharides

Ginseng polysaccharides (GPs), which are extracted from *Panax ginseng*, have been demonstrated to govern the gut microbes, enhance intestinal metabolism, and modify immunomodulatory effects [73]. A combination regimen with GPs and an αPD-1 mAb was found to improve the ratio of CD8+/CD4+ T cells and downregulate FoxP3 Treg cells in both tumor and peripheral tissues [74]. Additionally, the anti-tumor immunological response is improved by an increase in SCFA, valeric acid abundance, imbalances of indoleamine 2,3-dioxygenase (IDO) activity, and an increase in L-tryptophan production and a decrease in L-kynurenine production in addition to the kynurenine/

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tryptophan ratio. In addition, there was a notable rise in the number of *Bacteroides*, particularly *B. vulgatus* and *P. distasonis*. These findings suggested that the combo therapy might change the microbiota of the gut towards NRs to Rs in order to increase sensitivity of αPD-1 mAb treatment. Consequently, GPs and αPD-1 mAb could be a novel way to introduce anti-PD-1 immunotherapy to patients with non-small cell lung cancer(NSCLC).

Diosgenin

Diosgenin, a naturally occurring steroidal saponin, has anticancer and immunomodulatory properties and is derived from the genus Dioscorea [75, 76]. An experiment revealed that the co-administration of *diosgenin* and PD-1 mAb exacerbated tumor necrosis and apoptosis while also improving tumor growth inhibition [77]. The anti-melanoma impact of *diosgenin* appeared to be dependent more on immunity against tumors than on direct tumor suppressive activity, as demonstrated by the tumor tissues' clear production of IFN-γ and CD4$^+$/CD8$^+$ T-cell infiltration. *Diosgenin* may also improve the composition of the gut microbiota. Following the administration of *diosgenin*, there was a notable increase within the abundance of the *Sutterella* and *Lactobacillus* genera of the *Proteobacteria phylum* and the *Bacteroides* genus from the *Bacteroidetes phylum*, respectively. Nevertheless, CD4$^+$/CD8$^+$ T- cell infiltration and IFN-γ expression were both reduced when the gut microbiota was disrupted by an antibiotic cocktail technique. This proved that the immune response against tumor induction mediated by *diosgenin* involved the gut microbiota as a mediator.

Camu-camu

By controlling the gut microbiota, the Amazonian fruit known as *Camu-camu* (CC) has been demonstrated to have prebiotic benefits that protect mice from obesity and associated metabolic diseases [78]. The primary bioactive ingredient of *CC-Polyphenol* with anticancer action is *polyphenol castalagin*, which is abundant ellagitannin [79]. *Castalagin* affects the composition of the microbiota in a dose-dependent way and avoids αPD-1 resistance in mice given by FMT from NR patients with NSCLC. It was discovered [80] that *castalagin* treatment can reduce *Lachnosclostridium* and enhance the proportions of *Alistipes*, *Ruminococcus*, *Paraprevotella,* and *Christensenellaceae R-7* group. *Castalagin* may stimulate taurine-conjugated bile acids, downregulate secondary taurinated bile acids, and raise the CD8$^+$/CD4$^+$FOXP3 T-cell ratio when paired with isotype control for αPD-1 (IsoPD-1). It directly engages with *Ruminococcus*'s cellular envelope, promoting an anticancer reaction.

Inonotus hispidus (Bull.: Fr.) P. Karst

The annual wood rot medicinal fungus *Inonotus hispidus* (Bull.: Fr.) P. Karst., commonly referred to as "*Sanghuang*," is found in the temperate zone and gets its name from parasitic mulberry trees [81]. The spore powder (IHS) of this fungus contains polyphenols and triterpenoids that have a variety of pharmacological effects [82]. According to reports [83], IHS can decrease the abundance of *Allobaculum* and *Mucispirillum* while increasing its prevalence to *Oscillospira*, *Odoribacter*, *Rikenella*, *Dehalobacterium*, and *Coprococcus*. The IHS treatment increased the amount of L-arginine (L-Arg), which is beneficial for CD8$^+$ T cells [84]. This suggests that the gut microbiota plays a significant role in the anti-CRC advantages associated with IHS. Additionally, it has the ability to control the immune function mediated by the gut microbiota, namely through increasing the number of CD8$^+$ T cells and demonstrating anti-CRC characteristics. Therefore, IHS can regulate the gut microbiome and body immunity, and has anticancer effects, which is a potential drug choice for enhancing anti-tumor immune response by regulating the gut microbiome in the future.

Icariside I

Natural plant flavonoids called *Icariside I*, which were isolated from *Epimedium*, have a range of biological actions, including anti-tumor properties [85, 86]. *Icariside I*'s immunological anti-tumor efficacy was further reinforced by a notable increase in a number of lymphocytes, involving CD4$^+$ T, CD8$^+$ T, NK, and NKT cells, demonstrating an improvement in host immune function. It is commonly known that CD4$^+$ and CD8$^+$ adaptive T cells, among others, may trigger cytotoxic responses for anti-tumor immunotherapy [87]. Probiotics like *Lactobacillus* and *Bifidobacterium spp.* are significantly increased in response to *Icariside I* treatment, and their ability to promote antitumor immunity and enhance the activity of various lymphocyte subsets has been shown to suppress tumor growth [88]. Additionally, there was an increase in metabolites such as SCFAs and indole derivatives, which have been demonstrated to have an immunoregulation function and which contribute to the regulatory of host metabolism via colonic Gpr41/Gpr43 and AhR signaling [89, 90]. According to this study, *icariside I* regulates the gut microbiota and the metabolites that are produced from it, thereby enhancing the individual's gut barrier and immunity. In order to boost anti-tumor immune responses in the future, more research on this medication option is required.

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Safflower yellow

Carthamus tinctorius, a traditional Chinese medicine, contains *safflower yellow* (SY), the primary active component that has been identified and shown to have antitumor biological activity [91]. According to the study, SY and cyclophosphamide (CTX) together shown enhanced therapeutic efficiency in preventing the occurrence of liver tumors and extending the survival of mice with DEN-induced HCC. In the hepatocytes of the DEN-induced HCC mice model [92], SY inhibited the generation of inflammatory factors and reduced liver damage whether taken alone or in combination with CTX. Furthermore, SY can enhance the immunological milieu in DEN-induced HCC by promoting CD8$^+$ T cell infiltration. Additionally, SY reduced the number of *Bacilli* and *Alphaproteobacteria* and raised that of *Bacteroides* and *Erysipelotrichia*. It has been documented that *Bacteroides* is linked to several immunological and inflammatory systemic indicators [93]. These findings showed that SY may control the amount of gut microbiota linked to inflammation, which would control the tumor immune milieu. It is evident that SY has the ability to impact tumor immunology and intestinal microbiota, making it a viable option to increase the rate at which immunotherapy works.

Ganoderma lucidum

An important mushroom called *Ganoderma lucidum* (*G. lucidum*) has the potential to lower obesity in mice by altering the makeup of the microbiota in the gut and dramatically increase the activation of NK cells in patients with metastatic cancer [94, 95]. An extract obtained from *G. lucidum* (ESG) sporoderm-breaking spores shown immunoregulation and anticancer potential [96, 97]. The two immune checkpoints, CTLA-4 and PD-1, were both clearly downregulated by ESG. In the peripheral bloodstream in the tumor-bearing mouse, ESG also significantly increased the population of cytotoxic T cells (Tc) and the ratio of Tc to helper T cells (Th). These findings suggest that ESG could effectively restore the T cell paradigm from recuperating exhausted position through the suppression of co-inhibitory checkpoints. Additionally, ESG positively impacted the gut microbiome community, boosting the genera related to immunocompetence (*Helicobacter*, *Rikenella*, and *Turicibacter*) and decreasing some related to immunosuppression (Bacteroides), which may enhance CD8$^+$ T cell-mediated immunity [34]. The root cause of ESG likely played a role in suppressing co-inhibitory signaling (PD-1 and CTLA-4) and restoring fatigued Tc cells, of which the gut microbiome remodeling was an important factor [98]. As a result, ESG can be utilized as a potential natural product for subsequent studies to adjust the gut microbiome and

enhance the effectiveness of immune therapy by regulating the microbes. One of the most widely recognized *G. lucidum*-related compounds is oil of spores (GLSO). It was shown that GLSO can control the immune system by increasing NK cell cytotoxicity and macrophage phagocytosis in mice. A number of microbe genera and species, including *Lactobacillus*, *Turicibacter*, and *Romboutsia*, as well as *Lactobacillus intestinalis* and *Lactobacillus reuteri*, were found to be more abundant when GLSO was used. On the other hand, *Staphylococcus* and *Helicobacter* were found to be less abundant. These findings led to the regulation of a number of important metabolites, including dopamine, prolyl-glutamine, pentahomomethionine, leucyl-glutamine, L-threonine, stearoylcarnitine, and dolichyl β-D-glucosyl phosphate [99]. Therefore, GLSO can strengthen the immunological system of the body through the microbial metabolic axis, and is one of the potential natural products of anti-tumor immunotherapy.

Ginsenoside Rk3

With a lower molecular weight, *Ginsenoside Rk3* is produced from g*insenoside Rg1*. Previous research has demonstrated the antiapoptotic and anticancer effects of *ginsenoside Rk3* [100, 101]. *Ginsenoside Rk3* has been shown to be useful in establishing a healthy gut microbiota, which in turn can abrogate the invasion of gut macrophages, regulate immunological function, promote apoptosis in hepatocellular carcinoma, and prevent the growth of cancer. It effectively improved the amount of *Lactobacillus*, *Oscillibacter*, *Bacteroidetes*, *Lachnospiraceae*, *Bifidobacteriaceae*, *Akkermansia*, and *Bifidobacteriaceae* [102]. Additionally, it dramatically decreased the percentage of dangerous bacteria, including *Helicobacter*, *Ruminococcaceae*, and *Firmicutes*. Additionally, *ginsenoside Rk3* can downregulate inflammatory markers IL-17, IL-18, IL-22, and IL-6 linked to ILC3 and Th-17 [103], as well as directly boost the activities of *Akkermansia muciniphila* and glutamine concentration in *Akkermansia muciniphila* medium. Through immune system stimulation and gut microbiota regulation, it has anti-tumor effects. Additionally, as a possible natural product that modifies the microbes in the gut to improve the response of anti-tumor immunotherapy (Table 1).

Chinese native medicine compound prescription
Gegen Qinlian decoction

One widely recognized kind of classical TCM is *Gegen Qinlian decoction* (GQD). It can significantly lower inflammation and oxidative stress while also preventing kidney cancer in humans from developing [104–106]. Lv al. discovered that anti-mouse PD-1 combo therapy

Table 1 Natural products regulate DRTI through gut microbiota

Natural products	Agents	Cancer type	Targets	Effects of agents on gut microbiome	Immune regulation mechanism
Soluble fiber	Pectin	CRC	PD-1	Lacto bacillaceae, Bifidobacteriaceae, Erysipelotrichaceae, and Ruminococcaceae↑	Promote T cell infiltration and elicite CD8+ T cell
Asteraceae	Inulin	CRC	PD-1	Akkermansia, Lactobacillus, Roseburia hippurate, 1-methyl-L-histine, 3-methyl-L-histine, L-arginine, and oleate↑	Enhance the infiltration of CD8+ T cells and promote the differentiation of stem cells into Tcf1+PD-1+CD8+ T cells
Jujube	Ultrafine jujube powder (JP)	Colon cancer	PD-L1	Lachnospiraceae, Lactobacillaceae, Ruminococcaceae, SCFA↑	Increase CD8+ T-cell infiltration
Bilberry	Anthocyanins	Colon adenocarcinomas	PD-L1	Clostridia and Lactobacillus johnsonii and SCFAs↑	Enhanced CD8+ T cell infiltrations, butyrate↑
Ginseng	Ginseng polysaccharides	NSCLC	PD-1/PD-L1	B. vulgatus and P. distasonis↑	Activating CD8+ T cells and suppressing the function of tregs
Ginseng	Ginsenoside Rk3	hepatocellular carcinoma	–	Bacteroidetes, Lachnospiraceae, Bifidobacteriaceae, Akkermansia, Lactobacillus, Oscillibacter, Akkermansia muciniphila↑; Firmicutes, Helicobacter, Ruminococcaceae↓	IL–17, IL–18, IL–22, and IL–6↓ glutamine↑
Dioscorea	Diosgenin	Melanoma	PD-1	Lactobacillus genus, Sutterella genus↑, Bacteroides↓	CD4+/CD8+ T-cell infiltration and IFN-γ expression↑
Camu-camu	Castalagin	Fibrosarcoma, mammary adenocarcinoma	PD-1	Ruminococcus, Alistipes, Christensenellaceae R-7 group and Paraprevotella↑ Lachnosclostridium↓	CD8+/CD4+FOXP3 T-cell ratio↑
Gegen, Huangqin, Huanglian	Gegen Qinlian decoction (GQD)	CRC	PD-1	Bacteroidales Acidifaciens↑	CD8+ T cells, IFN-γ↑
Inonotus hispidus (Bull.: Fr.) P. Karst	Inonotus hispidus (Bull.: Fr.) P. Karst. spore powder	CRC	–	Oscillospira, Odoribacter, Rikenella, Dehalobacterium, Coprococcus↑ Allobaculum, Mucispirillum↓	Enhance CD8+ T cells infiltration
Epimedium	Icariside I	Melanoma	–	Lactobacillus spp. and Bifidobacterium spp.↑	CD4+ T, CD8+ T, NK, and NKT cells↑
Carthamus tinctorius	Safflower yellow	Liver tumour	–	Bacteroides, Erysipelotrichia↑ Bacilli, Alphaproteobacteria↓	Enhance CD8+ T cells infiltration
Ganoderma lucidum	Sporoderm-breaking spores of G. lucidum	Breast cancer	PD-1 CTLA-4	Helicobacter, Rikenella, and Turicibacte↑, Bacteroides↓	Increase the Tc ratio and Tc/Th ratio
Ganoderma lucidum	The oil of G. lucidum spores	–	–	Lactobacillus, Turicibacter, Romboutsia↑ Staphylococcus, Helicobacter↓	macrophage phagocytosis, NK cell cytotoxicity↑
Rheum officinale Baill., Prunus persica (L.) Batsch and Eupolyphaga sinensis Walker	Xiayuxue decoction	Hepatocellular carcinoma	–	Bacteroides and Lactobacillus↑	CXCR6+NKT cell↑

Table 1 (continued)

Natural products	Agents	Cancer type	Targets	Effects of agents on gut microbiome	Immune regulation mechanism
Coix Seed, aconite, herba patriniae	*Yi-Yi-Fu-Zi-Bai-Jiang-San*	CRC	–	*Bifidobacterium, Prevotellaceae, Lactobacillus, Ruminococcaceae, Clostridium, Lactobacillus rhamnosus* and *Clostridium butyricum*↑ *Bacteroides, Lachnospiraceae,* unclassified lachnospiraceae, enterotoxigenic *Bacteroides fragilis* mRNA↓	$CD4^+, CD25^+$ Foxp3 positive Treg cells, IL-6, IL-17A, RORγt, TNF-α↓
Astragali Preparata, Atractylodes, Coicis Semen, Agrimonia pilosa Sparganii Rhizoma, Rhizoma Curcumae, Sophorae Flavescentis Radix, and Coptidis Rhizoma	*Wenzi Jiedu* Recipe	CRC	–	*Oscillibacter, Bacteroides_acidifacien*↑ *Bacteroidales_bacterium*↓	$CD8^+$ T cells, IL-10, IFN-g, and TNF-a↑
Fuzi, Zhigancao, and Ganjiang	*Sini* Decoction	CRC	–	*Bacillus coagulans, Lactobacillus, Bifidobacterium*↑ *Bacteroides fragilis,* Sulphate reducing bacteria↓	$CD8^+$ T cells↑ $CD4^+$ T cells, IL-6, IL-17, TNF-α, IFN-γ↓
Medlar, raspberry, Epimedium, Psoralea, Duchesneau indica, dodder, Pinellia ternate, Radix puerariae, Radix scutellaria, mulberry, Prunella spica, Rattan pear root, and Rehmannia glutinosa	Chinese herbal medicine anticancer cocktail soup	CRC	–	*Turicibacter, Faecalibaculum, Lactobacillus*↑ *Coriobacteriaceae_UCG002, Dubosiella, Lachnospiraceae_NK4A136, Lachnospiraceae*↓	Th17, $CD8^+$ T cells, and NK cell↑

and GQD treatment significantly slowed the expansion of the tumor [107]. Additionally, the combination therapy markedly raised the percentage of CD8$^+$ T cells and IFN-γ expression, both of which are essential components of anti-tumor immunotherapy. Combination therapy has also been shown to downregulate PD-1 and raise IL-2 levels, indicating that GQD may successfully reduce inhibitory checkpoints and rebuild T-cell activities. In addition to controlling the gut microbiome, GQD has been shown to enhance the number of LysoPC, vignatic acid B, LysoPE, LysoPE, and PI metabolites, all of which may strengthen the immune system. *Bacteroidales Acidifaciens* is one of the primary microbes that promotes IgA production in the intestinal tract [108, 109]. It was demonstrated that GQD controls the glycerophospholipid and sphingolipid metabolic pathways, amplifying the impact of PD-1 inhibition in CRC.

Xiayuxue decoction

Prunus persica (L.) Batsch, *Eupolyphaga sinensis Walker*, and *Rheum officinale Baill* are the three herbal products that make up the TCM compound formula known as *Xiayuxue* decoction (XYXD). Many pharmacological effects are demonstrated by XYXD, such as anti-tumor, anti-fibrosis, anti-inflammatory, gut microbiota regulation, and immune response modulation [110–112]. It was shown [113] that through modifying the relationship between gut microbiota and bile acids, XYXD can enhance the immunological impact of NKT cells against hepatocellular carcinoma (HCC). The primary process was augmenting the quantity of *Bacteroides* and *Lactobacillus* to facilitate the synthesis of bile salt hydrolase (BSH). By reducing the amount of *Eubacterium*, which lowers the transformation of main bile acids to secondary bile acids, BSH increases the level of primary bile acids and turns conjugated bile acids into primary bile acids. Primary bile acids cause the liver's NKT cells to release interferon-γ, which has anti-HCC immunological effects. Thus, by controlling the gut microbiota, XYXD is anticipated to be a potent natural medication ingredient that can improve the effectiveness of the immune system's response to tumors.

Yi-Yi-Fu-Zi-Bai-Jiang-San

A thousand-year-old *Huangdi Neijing* prescription known as *Yi-Yi-Fu-Zi-Bai-Jiang-San* (YYFZBJS) is frequently employed by TCM for treating problems with the digestive tract [114, 115]. It was discovered that YYFZBJS therapy for intestinal lymphatic and mesenteric lymph nodes (MLN) reduced the amount of accumulated CD4$^+$ CD25$^+$ Foxp3 positive treg cells in ApcMin/+mice. The modified tregs caused by YYFZBJS also decreased the phosphorylation of β-catenin and

inhibited the growth of CRC cancer cells. Subsequent research revealed that the complex microbiome is primarily responsible for this antitumor effect. Following YYFZBJS treatment, the prevalence of various probiotic genera significantly increased, including *Bifidobacterium* and *Prevotellaceae*, and nearly eliminated others, as *Bacteroides*, *Lachnospiraceae*, and Unclassified *Lachnospiraceae* [116]. Additionally, YYFZBJS can upregulate *Lactobacillus rhamnosus* (LGG) and *Clostridium* butyricum and raise the associated prevalence of pathogenic microbes, including *Lactobacillus*, *Ruminococcaceae*, and *Clostridium* [117]. Following YYFZBJS intervention, enterotoxigenic *Bacteroides fragilis* (ETBF) was shown to have reduced mRNA expression. Furthermore, YYFZBJS suppresses HIF-1α expression, enhances immune system response in *vivo* [118], causes treg cells to accumulate, modifies the function of nTreg cells that are naturally present, and significantly lowers the levels of the proteins IL-6, IL-17A, RORγt, and TNF-α. Consequently, YYFZBJS is a viable intervention tool for sensitizing and anti-tumor immunotherapy since it may simultaneously modulate gut microbiota and improve immune response.

Wenzi Jiedu recipe

Clinical evidence has demonstrated the efficacy of TCM formula Wenzi Jiedu recipe (WJR) in the treatment of CRC. Astragali Preparata, Atractylodes, Rhizoma Curcumae, Agrimonia pilosa Sparganii Rhizoma, Sophorae Flavescentis Radix, and Coptidis Rhizoma are among its contents. According to a study, WJR could regulate the immune system and gut microbiota to prevent the development of CRC [119]. WJR may, on the one hand, boost the percentage of CD8$^+$ T cells and the production of TNF-a, IFN-g, and IL-10, three immune-associated cytokines. On the other hand, WJR may reduce harmful bacteria and raise helpful ones. Following WJR therapy, there is a drop in the amount of uncultured Bacteroides acidifacien and an increase in Oscillibacter and Bacteroides acidifacien. The anti-tumor immunotherapy response to WJR sensitization, however, has not been studied. WJR has the ability to stimulate the immune response to treat tumors through a variety of processes, including the modulation of the gut microbiome, the development of immune cells and immunologically linked proteins.

Sini decoction

Fuzi, *Zhigancao*, and *Ganjiang* are the three ingredients of *sini* decoction (SND), a traditional TCM remedy found in "*Shang Han Lun*." To treat CRC, SND extract can increase the expression of CD8$^+$ T lymphocytes and occludin in the colonic mucosal layer while inhibiting the expression of CD4$^+$ T cells and TNF-α, IL-6, IL-17,

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and IFN-γ in CRC tissue. Furthermore, SND can successfully interfere to the progression of CRC by modifying the composition of the gut microbiome by increasing the abundance of *Bifidobacterium*, *Lactobacillus*, and *Bacillus coagulans*, while decreasing *Bacteroides fragilis* other *Sulphateducing* microbes [120].

The composition of the intestinal microbiota in mice, enhanced by SND, influences the development and course of malignancies through immune system control. It may help to sensitize the immune system against cancers by regulating the gut microbiome.

Chinese herbal medicine anticancer cocktail soup

Prof. Te Liu of the *Shanghai* Geriatric Institute of Chinese Medicine and *Shanghai* University of Traditional Chinese Medicine created the Chinese herbal medicine anticancer cocktail soup (CHMACS), which was mostly made of extracts from 14 Chinese herbal products. CHMACS was shown to alter the gut microbiome-Th17 axis, which in turn activates immune cells and destroys cells. In CRC mice, CHMACS markedly increased Th17, CD8^{+}, and NK cell activation, indicating increased immunological activity. In the intestinal microbiota of mice harboring tumors, it also boosted the abundance of *Turicibacter*, *Faecalibaculum*, and *Lactobacillus* while drastically lowering the amount of *Coriobacteriaceae_UCG002*, *Dubosiella*, *Lachnospiraceae*_NK4A136_group, and *Lachnospiraceae* [121]. Further research is necessary to determine the clinical use of CHMACS, which can improve anti-tumor immunity and control gut microbiota. This may play a therapeutic part in anti-tumor immunotherapy sensitization.

Discussion

Across the past decade, there has been a notable advancement in the field of cancer immunotherapy. At the moment, PD-1/PD-L1 inhibitors and CTLA-4 inhibitors are receiving the greatest interest. These inhibitors mainly target B7/CTLA-4 and PD-1/PD-L1, two immunological checkpoints that are critical for T cell activation. Immune checkpoint inhibitors stimulate the differentiation and proliferation of T cells, improve the function of CD8^{+} T cells, activate the body's autoimmune function, and enhance the recognition and cytotoxicity function of dendritic cells on tumor cells or tissues by triggering natural killer (NK) cells in mononuclear macrophages. Maintain the tumor-immune cycle, activate immunological brake responses and successfully block tumor immune escap [122–127]. There are noteworthy differences in response rates among various cancer types, despite the fact that many individuals show a notable response and long-lasting effectiveness to immunotherapy. For instance, people with Hodgkin's lymphoma

that is refractory may respond at a rate higher than 80%. On the other hand, patients with CRC that is proficient in mismatch repair show very little response [128, 129], and the total response rate in tumor immunotherapy varies from 20 to 40% [10]. Tumor immunotherapy's low response rate is therefore still a serious problem that needs to be solved.

The organism's intestinal microbiota is intimately connected to the immune system and is regarded as its second genome. Resistance to tumor immunotherapy may result from an imbalance in the gut microbiota and metabolites. Therefore, predicting the response of immune drugs effectively to avoid ineffective treatments and enhancing the response rate of patients to immunotherapy are major challenges in the field of tumor immunotherapy. It is well known that deviations in the WNT-β-catenin [130], MAPK [131], JAK/STAT [132], PI3K-AKT-mTOR [133], and Transforming growth factor-β signaling pathways are the primary causes of resistance to anti-tumor immunotherapy strategy [134]. And abnormalities also involve gene mutations in PTEN [135], SIRPA [136], TBK1 [137], the Y chromosome [138], as well as immune cells and stromal cells such as Treg [139], MDSCs [140], TAMs [141], CAFs [142], loss of tumor immunogenicity [143], abnormalities in Indoleamine 2,3-dioxygenase [144], Ubiquitin-specific protease 12 (USP12) [145], and Hypoxia [146]. Furthermore, it was found that the gut microbiota, which includes the bacteria *F. nucleatum*, *Coprobacillus cateniformis*, *Akkermansia muciniphila*, *Bifidobacterium*, *Ruminococcaceae*, *Faecalibacterium*, *Phyla Firmicutes*, and *Actinobacteria*, in addition to their metabolites, such as peptidoglycan hydrolase secretes antigen A, trimethylamine N-oxide, short-chain fatty acids, inosine, succinic acid, and L-arginine, are closely linked to the process of sensitizing the effectiveness of anti-tumor immunotherapy. In terms of treatment, *Pectin, Inulin, Jujube, Anthocyanins, Ginseng polysaccharides, Diosgenin, Camu-camu*, and *Inonotus hispidus* (*Bull.Fr. P. Karst, Icariside I, Safflower yellow, Ganoderma lucidum*, and *Ginsenoside Rk3* are natural active ingredients. Furthermore, a number of Chinese native medicine compound prescriptions have demonstrated impressive therapeutic results, including the *Sini* decoction, *Yi-Yi-Fu-Zi-Bai-Jiang-San, Gegen Qinlian* decoction, *Xiayuxue* decoction, *Wenzi Jiedu* recipe, and Chinese herbal medicine anticancer cocktail soup. Through the regulation of the microbiota and its metabolites, they may improve the effectiveness of anti-tumor immunotherapy.

The present study shows that natural products may alter the gut microbiome, which has important implications. In particular, these products decrease the frequency of dangerous bacteria, *Firmicutes*, *Helicobacter*,

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Ruminococcaceae, and others, while concurrently increasing the quantity of helpful probiotics, such as *Lactobacillaceae*, *Bifidobacteriaceae*, *Erysipelotrichaceae*, and *Ruminococcaceae*. Additionally, the study highlights the regulation of key intestinal microbial metabolites, such as butyrate, SCFAs, IDO, L-tryptophan, and L-kynurenine. These metabolites increase immune cell infiltration, which improves the system's reaction towards immunotherapy. Additionally, the research delineates plausible pathways via which natural compounds could potentially enhance the immune system's reaction to treatment. However, current research is mainly focused on CRC, NSCLC, hepatocellular carcinoma, melanoma, fibrosarcoma, and mammary adenocarcinoma, which has certain limitations. In addition, natural products exhibit multi-target and multi-pathway intervention effects. They enhance the efficacy of immunotherapy by regulating intestinal microbes and their metabolites. It did not, however, concentrate on the only regulatory mechanism, and there are no particular metabolites or microbes that can be used to forecast how well immunotherapy will work. Natural products are also expected to be considered as promising immunotherapy sensitizers for various malignant tumors, such as pancreatic cancer, esophageal cancer, and stomach cancer in the future.

Furthermore, we found that the natural active ingredients with the effect of invigorating *Qi* (*Jujube, Anthocyanins, Ginseng polysaccharides, Diosgenin, Icariside I, Ganoderma lucidum, Ginsenoside Rk3*) and promoting blood circulation(*Inonotus hispidus(Bull.: Fr.) P. Karst, safflower yellow* and *Xiayuxue* decoction) may be more dominant in the effect of sensitizing anti-tumor immunotherapy. The 2024 Nature Cancer study found [147] that co-stimulation of immune presenting cells and antigens associated with the tumor was essential for the substantial introduction of tumor-specific CD8$^+$ T cells into the tumor microenvironment. This was determined by continuously monitoring the dynamic changes in the differentiation and proliferation of tumor-specific CD8$^+$ T cells. There can be difficult to recruit CD8$^+$ T cells into the tumor microenvironment if the antigen-presenting cells do not sufficiently convey the tumor antigen to the CD8$^+$ T cells. As such, they are unable to multiply quickly enough to produce a potent anti-tumor reaction. Thus, the primary mechanism of immunotherapy resistance is a deficiency of T cells [148]. In TCM, *Qi* serves a physiological purpose akin to that of T cells in contemporary medicine. Their respective roles include controlling the body's immunity and defending against foreign infections. *Qi*-tonifying medications can boost T cell counts, which improves sensitization and encourages the immune system to fight cancer. A study that was published in Clinical Cancer Research in 2023 discovered

[149] that heparin medication given in conjunction with anti-PD1 or adoptive cell transfer (ACT) produced combinatorial effects against cancer. These benefits have been attributed, possibly in a part, with tumor vasculature normalizing, which decreased the infiltration of treg while accelerating M1 macrophage polarization, ultimately leading to higher anticancer T-cell responses. Furthermore, a clinical trial [150] examining the combined use with the PD-1 immune checkpoint inhibitor carrilizumab and apatinib among individuals with advanced NSCLC who already received treatment with chemotherapy demonstrated a noteworthy clinical improvement for patients harboring STK11/KEAP1 mutations (The wild type, ORR is 28.1% and one-year survival is 53.1%; The mutation, ORR is 42.9% and one-year survival is 85.1%). The reason for the continuation of the Phase III trial (NCT04203485) may be because proper reduction of angiogenesis can improve PD-1/PD-L1 blocking through decreasing tumor-associated macrophage recruitment, boosting CD8$^+$ T cell infiltration, and alleviating hypoxia. Blood-activating medications in traditional Chinese medicine are as effective as heparin and anti-angiogenic medications in contemporary medicine. Studies have indicated [151, 152] that natural products with blood-activating effects can facilitate the normalization of tumor blood vessels and inhibit tumor angiogenesis. Therefore, natural blood-activating drugs may enhance the response to anti-tumor immunotherapy by regulating tumor blood vessels.

The 2023 Cancer Cell study [153] demonstrated particular expression of flaky collagen and validated a substantial rise in fibroblasts after PD-1 therapy. In 2021, Nature study showed [154] that the discoidin domain receptor 1 (DDR1) performs a vital function in preventing immune cells in accessing the tumor site. By doing this, you can help surround the tumor with a physical barrier that prevents T cells for entering it and eliminating cancerous cells. Therefore, in addition to CD8$^+$ cell depletion inhibiting the tumor immune response, immunotherapy induces a significant increase in tumor-associated fibroblasts, forming a spatial immune barrier to impede T cell infiltration. Although T cells are abundant, they are unable to penetrate the tumor microenvironment to carry out their functions, resulting in a diminished tumor immune response. Supplementing *Qi* and promoting blood circulation, as representative treatment methods in traditional Chinese medicine, may offer therapeutic benefits to patients with low responsiveness to T cell depletion immunotherapy by supporting qi supplementation and boosting immune function. By reducing metabolites and removing harmful gases, activating blood drugs may offer therapeutic benefits to patients with a low response to immunotherapy that inhibits

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T cell infiltration. However, the differences in natural products from various sources, types, and functions in primary and secondary drug resistance in anti-tumor immunotherapy still need further exploration.

In the future, studies on immunotherapy resistance should focus on predicting patients with primary and secondary resistance based on various factors, including demographic information, tumor stages, pathological types, biological indicators, and other relevant aspects. This approach will help identify the groups that will benefit most from immunotherapy, ultimately leading to more individualized and precise treatment [155]. Nevertheless, as the immune response's regulatory systems are intricate, the effectiveness and potential benefits to immunotherapy cannot be entirely predicted by simply one biomarker. Therefore, multiple biomarkers should be integrated in clinical applications. It is essential to develop a quantifiable predictive model for assessing the immune response. Utilizing a big data platform and leveraging artificial intelligence, aiming to construct a visual decision tree model characterized by high specificity, sensitivity, and accuracy, which will provide a reliable foundation for evaluating the predictive value of potential biomarkers [156]. In addition, biological samples such as pathological biopsies, peripheral blood, urine, and stool from immunoresistant patients can be obtained through the open biobank. By identifying particular targets and clarifying the mechanisms underlying immunoresistance [157, 158], new technologies such as single-cell sequencing and spatial ecology can open the door to the eventual clinical creation of new drugs.

Synthetic biology, medicine, and other fields are coming together to create novel drug delivery platforms and enhance existing medication processes, such as engineered bacteria, intestinal chips, and biofilm nanomedicine delivery systems, has gradually permeated clinical research and foundational studies on gut microbiota [46]. This advancement is driving progress in gut microbiota research, with the ultimate goal of identifying specific intestinal microorganisms or metabolites. It may boost the effectiveness of immunotherapy and turn patients with tumors who are refractory or secondary to tumor immunosuppressive therapy into patients who respond well to treatment. With the use of synthetic materials and modified microbes, immunotherapy may take a new turn in the future. Additionally [159], the application of small molecules from natural products and new polymer materials will also contribute to advancements in immunotherapy. The future trajectory for treating tumor immunodrug resistance may involve tailored multi-omics analysis in conjunction using personalized medicine, given the multiplicity of contributing factors and individual differences among the symbiotic microbiome.

Availability of data and materials
No data was used for the research described in the article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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