Emerging role of natural products in cancer immunotherapy

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Abstract Cancer immunotherapy has become a new generation of anti-tumor treatment, but its indications still focus on several types of tumors that are sensitive to the immune system. Therefore, effective strategies that can expand its indications and enhance its efficiency become the key element for the further development of cancer immunotherapy. Natural products are reported to have this effect on cancer immunotherapy, including cancer vaccines, immune-check points inhibitors, and adoptive immune-cells therapy. And the mechanism of that is mainly attributed to the remodeling of the tumor-immunosuppressive microenvironment, which is the key factor that assists tumor to avoid the recognition and attack from immune system and cancer immunotherapy. Therefore, this review summarizes and concludes the natural products that reportedly improve cancer immunotherapy and investigates the mechanism. And we found that saponins, polysaccharides, and flavonoids are mainly three categories of natural products.

KEY WORDS Natural products; Cancer immunotherapy; Immunosuppressive microenvironment; Cancer vaccines; Immuno-check points; Adoptive immune-cells transfer immunotherapy

Abbreviations: AKT, alpha-serine/threonine-specific protein kinase; B2M, beta-2-microglobulin; bFGF, basic fibroblast growth factor; BMDCs, bone marrow dendritic cells; BPS, basil polysaccharide; BTLA, B- and T-lymphocyte attenuator; CAFs, cancer-associated fibroblasts; CCL22, C–C motif chemokine 22; CIKs, cytokine-induced killer cells; COX-2, cyclooxygenase-2; CRC, colorectal cancer; CTL, cytotoxic T cell; CTLA-4, cytotoxic T lymphocyte antigen-4; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; FDA, US Food and Drug Administration; HCC, hepatocellular carcinoma; HER-2, human epidermal growth factor receptor-2; HIF-1α, hypoxia-inducible factor-1α; HMGB1, high-mobility group box 1; HSPs, heat shock proteins; ICD, Immunogenic cell death; ICTs, immunological checkpoints; IFN-γ, interferon-γ; IL-10, interleukin-10; LLC, Lewis lung cancer; Mel-1, myeloid leukemia cell differentiation protein 1; MDCs, myeloid-derived suppressor cells; MHC, major histocompatibility complex class; MITF, melanogenesis associated transcription factor; MMP-9, matrix metalloprotein-9; mTOR, mechanistic target of rapamycin; NF-kB, nuclear factor kappalight-chain-enhancer of activated B cells; NKTs, natural killer T cells; NSCLC, non-small cell lung cancer; OVA, ovalbumin; PD-1, programmed death-1; PD-L1, programmed death receptor ligand 1; PGE-2, prostaglandin E2; PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TAMs, tumor-associated macrophages; TAP, transporters related with antigen processing; T cells; TGF-β, transforming growth factor-β; Th1, T helper type 1; TILs, tumor infiltration lymphocytes; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; Tregs, regulatory T cells; TSA, tumor specific antigens; VEGF, vascular endothelial growth factor.

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natural products, which reflected significant effects combined with cancer immunotherapy through reversing the tumor-immunosuppressive microenvironment. Besides, this review also collected the studies about nano-technology used to improve the disadvantages of natural products. All of these studies showed the great potential of natural products in cancer immunotherapy.

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1. Introduction

Cancer immunotherapy has become an irreversible trend herald in the field of cancer therapy and is regarded as the fourth type of anti-tumor treatment after surgery, radiotherapy, and chemotherapy due to the obvious efficacy and low side effects. This kind of breakthrough therapy is defined that regulates immunological response through activating the organism’s immune defense system or action of biological compounds to suppress and prevent tumor growth. Up to now, 7 types of immune-checkpoint inhibitors, 2 kinds of adoptive immune-cell immunotherapies, and 4 types of cancer vaccines for anti-cancer treatments have been approved by the US Food and Drug Administration (FDA, Fig. 1).

Despite cancer immunotherapy has achieved numerous remarkable advances so far, its indications still focus on several types of tumors that are sensitive to the immune system (also named as “hot tumor”), such as metastatic melanoma, urothelial carcinoma, and prostate cancer. For the tumor with poor immunogenicities (also named as “cold tumor”) like triple-negative breast cancer, colorectal cancer, and lung cancer, there is almost no significant efficiency of cancer immunotherapy. And this main reason resulting in this limitation is the immunosuppressive microenvironment within tumor sites, which can assist tumor cells in immune escape and even promote its growth. As shown in Fig. 2B, the hot tumors can normally express tumor-specific antigens and the immune system can distinguish these antigens and then effectively kill tumor cells with the assistance of immunotherapy. But for cold tumors, they will establish their unique immunosuppressive microenvironment against the recognition and attack from the immune system. And this microenvironment can effectively passivate cancer immunotherapy.

For example, although the number and activity of effective T cells (Teffs) can be increased and improved by cancer vaccines or immune-checkpoint inhibitors, the cold tumor cells still can weaken Teffs’ activities through immunosuppressive cells like regulatory T cells (Tregs) and immunosuppressive factors, such as interleukin-10 (IL-10), IL-13, IL-16, prostaglandin E2 (PGE-2) and so on. Therefore, effective strategies that can reverse and remodel the complex immunosuppressive microenvironment within tumors become the key element for cancer immunotherapy to expand its indications.

Natural products, which refer to the compounds extracted and/or optimized from nature, have shown great potential in the field of cancer immunotherapy due to their multi-target regulated abilities in recent years. It has been found that they can effectively enhance the therapeutic outcome and expand indications of all kinds of cancer immunotherapies, including cancer vaccines, immune-checkpoint inhibitors, and adoptive immune-cells transfer therapy. In addition, there are also some clinical trials about natural products combined with immunotherapy that have been approved by the FDA (Table 1). Besides, natural products have achieved huge success in the discovery of anti-tumor agents. About 47% of anti-tumor agents are derived from naturally occurring compounds. Because of the multibenefits of natural products on anti-tumor and immunomodulatory fields, it has shown great potential to become qualified adjuvants for tumor immunotherapy.

This review collects and generalizes the newly obtained studies about how natural products expand indications and enhance the efficiency of cancer immunotherapies through remodeling tumor immunosuppressive microenvironment. Firstly, the antagonistic relationship between tumor immunosuppressive microenvironment and current cancer immunotherapies has been elaborated. Next, as for the three types of tumor immunotherapies approved by the FDA, the information about how natural products improve these three treatments through multi-cells and multi-pathways moderating effects have been provided in this part. Then, the effective nano-drug delivery system designed to overcome the shortages of natural products also are exhibited and discussed. And this multi-targeted immune sensitized effect of natural products is summarized in the last part.

2. Tumor immunosuppressive microenvironment

For the current cancer immunotherapies, their efficiencies mainly depend on the function of Teffs, which play the central role in the anti-tumor effect of the immune system. However, the existence of the tumor immunosuppressive microenvironment weakens the Teffs’ activities. Therefore, a deep understanding of the immunosuppressive microenvironment will help to find the key elements for improving the efficiency of cancer immunotherapy. And this inhibition of Teffs induced by the immunosuppressive microenvironment in tumors can be concluded into three main aspects: poor immunogenicity, Immunological checkpoints, and immunosuppressive factors and cells.

2.1. Poor immunogenicity

The most significant basis for the elimination of tumor cells by the immune system is enough immunogenicity, which mainly depends on the number of tumor-specific antigens (TSA) that express on the surface of tumor cells (Fig. 2B). It is also the straightforward reason that results in limited clinical benefits of cancer immunotherapy for poorly immunogenic (cold) tumors compared to immunogenic (hot) tumors. TSAs are usually induced by tumor-specific mutations. The lack of antigenic mutations will cause the lack of recognition by T cells for tumor cells.

Moreover, tumor cells can also prevent the TSAs-presenting process to the surface of tumor cells by the restriction of major histocompatibility complex class (MHC) through altering some elements in the TSAs presenting procedure, including...
proteasomes and transporters related to antigen processing (TAP), beta-2-microglobulin (B2M) or MHC. Either lack of antigenic mutations or alterations in the antigen-presenting process may result in the “off-target” effect of T cell-based cancer immunotherapy.

2.2. Immunological checkpoints

Even though Teffs can recognize and bind to tumor cells, tumor cells still can utilize the immunological checkpoints (ICTs) to avoid the attack of Teffs (Fig. 2C). The ICT is the other important aspect of tumor cells for interrupting Teffs’ function. The cytotoxic T lymphocyte antigen-4 (CTLA-4) is the first explored immunological checkpoint in previous studies and named as the “brake” of Teffs responses. The affinity between it and B7 cos-timulatory molecules is much higher than that between CD28 and B7, and thus it can effectively terminate the activation of Teffs. The other immunological checkpoints, programmed death-1 (PD-1) and programmed death receptor ligand 1 (PD-L1), also can inhibit the activity of Teffs for tumor cells. The PD-L1 overexpression in tumor cells will also passivate the therapeutic outcomes of immunotherapy. And there are increasing the number of immune-check points, such as T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), B- and T-lymphocyte attenuator (BTLA), and Killer-cell immunoglobulin-like receptors (KIRs) have been reported in these years.

2.3. Immunosuppressive cells and factors

Besides poor immunogenicity and the inhibition of ICTs, the immunosuppressive cells and factors also limit the efficiency of cancer immunotherapy (Fig. 2D). Immunosuppressive cells mainly consist of tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), dendritic cells (DCs), natural killer T cells (NKTs), mast cells, and tumor invading lymphocytes. And these cells can weave an immunosuppressive network through the secretion of immunosuppressive factors, such as IL-10, PGE2, and transforming growth factor-β (TGF-β). Besides, some compounds in TME also can induce immunosuppression, such as H⁺, NO, and polyamine oxide.

Tumor cells can induce the non-degranulated mode of mast cells through secretion of H⁺, NO, chondroitin sulfate, and oxidized polyamines. And this mode can make mast cells promote the growth of the tumor and induce immunosuppression. In addition, the histamine, TGF-β, and IL-10 secreted from mast cells will inhibit activities of TAMs. Besides, mast cells also can affect the migration and function of DCs through secreting PGE2, indirectly enhancing the immunosuppression of tumors. In addition, MDSCs in tumor also will secret TGF-β and IL-10 to inhibit Teffs’ activities.

Moreover, tumor cells also secret macrophage-derived chemokines, C–C motif chemokine 22 (CCL22), to recruit monocytes into tumors and these monocytes will finally differentiate into TAMs under the condition of immunosuppressive factors. TAMs are also an important part of solid tumors and almost 40% of non-malignant cells are TAMs in some kind of tumors. These cells will transform into M2 immunosuppressive phenotype under the function of tumor-derived cytokines, including vascular endothelial growth factor (VEGF), galectin-1, gangliosides, TGF-β, PGE2, and IL-10. Meanwhile, IL-10, TGF-β, and VEGF will inhibit the antigen presentation process of DCs. Then, the IL-10 also can recruit Tregs, which is a key factor to maintain immune tolerance, and Tregs will induce Teffs lymphocyte lysis through several ways like secreting TGF-β. In addition, IL-13 secreted from NKTs within TME will also promote the secretion of TGF-β, thereby inhibiting the function of Teffs.

As mentioned above, it can be concluded that the tumor immunosuppressive microenvironment is a complex network within tumor sites to help tumor cells escape from the recognition and attack of the immune system, especially for the Teffs. Therefore, finding effective agents to remodel this unique microenvironment and reactivate Teffs’ function become the key element for cancer immunotherapy. Natural products have shown great potential in remodeling tumor immunosuppressive microenvironment to improve the therapeutic outcome of cancer immunotherapy.
3. Natural products expand indications and enhance the efficiency of cancer vaccines

The cancer vaccines, including cancer-preventive vaccines and cancer-therapeutic vaccines, belong to one of the cancer-specific active immunotherapies. Cancer-preventive vaccines mainly fight virus infection through the induction of specific antibodies and long-lived memory B lymphocyte cells. Some of them, especially for those containing attenuated pathogens, also can trigger cellular immunity. Except for HPV vaccines (GRADASIL® and CERVARIX®) are cancer-preventive vaccines, most cancer vaccines belong to therapeutic vaccines, such as PROVENCE®. These vaccines kill malignant cells mainly through inducing CD8+ cytotoxic T lymphocytes induced by TSAs.

The presenting processes of TSAs in cancer vaccines is a complex process, and the effect of cancer vaccines depends on the optimized combination of antigens, adjuvant, carrier, and vaccination routes. In addition to effectively activating CD8+ cytotoxic T lymphocytes, cancer vaccines must face the following two challenges: poor immunogenicity and tumor-associated immunosuppression.

3.1. Natural products enhance the tumor immunogenicity by inducing the immunogenic cell death (ICD) effect

Immunogenic cell death (ICD) effect is a kind of cell apoptotic pathways, which can induce cancer cell death routine mainly depend on damage-associated molecular patterns (DAMPs), such as calreticulin (CRT), heat shock proteins (HSPs), and high-mobility group box 1 (HMGB1), and markedly enhance the immunogenicity of these dying tumor cells (Fig. 3). ICD effect can make cancer cells into “therapeutic vaccines” that can induce anti-tumor immunity without any additional adjuvants.
Up to now, only a limited number of agents can induce ICD\textsuperscript{70}. Capsaicin, the pungent alkaloid of chili pepper, reportedly could trigger the ICD effect in primary effusion lymphoma (PEL) cells through the induction of DAMPs exposure, including HSP90 and calreticulin\textsuperscript{71} (Fig. 3A). Besides, it also could activate the DCs through binding to vanilloid receptor 1 (VR1)\textsuperscript{72}. And this counteraction effect of immune suppression on DCs in PEL microenvironments has been proved in this study. Moreover, capsaicin could induce apoptotic and pro-survival autophagy through reducing signal transducer and activator of transcription 3 (STAT3) phosphorylation, which depends on down-regulating the expression of Induced myeloid leukemia cell differentiation protein 1 (MCL-1), a kind of anti-apoptosis molecules. And similar results were also be found in several solid and hematological tumors\textsuperscript{73–76}.

Ginsenoside Rg3, a saponin from ginseng, is reported that could induce the significance of ICD both in immunogenic tumor-like melanoma (B16F10) and non-immunogenic tumor-like Lewis lung cancer (LLC, Fig. 3B)\textsuperscript{77}. The ICD effect induced by Rg3 could increase the expression of CRT and HSP60, which are chaperone proteins and ICD markers, on the surface of B16F10 and LLC cells. In addition, within the “eat me” signal of CRT, DCs took up the debris of dead tumor cells treated by Rg3 more easily than normal tumor cells. Furthermore, the Rg3-induced ICD effect could enhance interferon \(\gamma\) (IFN-\(\gamma\)) secretion to inhibit tumor growth.

Resveratrol, a non-flavonoid polyphenol widely distributed in the leaves and skins of grapes, also be verified that could induce ICD effect in ovarian carcinoma cells\textsuperscript{78} (Fig. 3C). And related studies showed that the cell surface exposure of CRT and HMGB1 were markedly increased in this kind of cell line. Besides, the effect of cancer vaccines of resveratrol has also been found in tumor rat models, in which subcutaneous injection of ID8 cells pre-treated by resveratrol could dramatically decrease the volume of tumors. Moreover, resveratrol also significantly inhibited the secretion of TGF-\(\beta\) while promotes the numbers of IL12 and IFN-\(\gamma\).

Zhang et al.\textsuperscript{79} found the injection of quercetin and alantolactone at a molar ratio of 1:4 could induce ICD in the microsatellite-stable colorectal cancer (CRC) model (Fig. 3D). Their results showed this combination could induce the number of

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**Figure 3** Immunogenic cell death (ICD) effect induced by natural products. Natural products (A. Capsaicin; B. Ginsenoside Rg3; C. Resveratrol; D. Quercetin: Alantolactone = 1:4; E. Shikonin) induce immunogenic cell death (ICD) effect through damage-associated molecular patterns (DAMPs), including calreticulin (CRT), heat shock proteins (HSPs), and high-mobility group box 1 (HMGB1), to increase the tumor immunogenicity and make the tumor cells into “therapeutic vaccines”.

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CRT exposed on the tumor cells member and HMGB1 release. In this combination, they found the injection of alantolactone alone could induce the ICD effect and quercetin could enhance this effect through modulating the production of reactive oxygen species (ROS) and interfering with protein kinase (also known as “AKT”) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways. Moreover, it also could inhibit the secretion of IL-10, TGF-β, IL-1β, and CCL2. And the long-term anti-tumor immune-memory effects of this combination also were found in this study.

Besides making tumor cells be “therapeutic cancer vaccines”, the ICD effect also can enhance the curative effect of DCs-based tumor vaccines. Shikonin, a natural tea quinone pigment from Lithospermum erythrorhizon, has been reported to be an adjuvant for DCs-based cancer vaccines via the induction of ICD effect (Fig. 3E). It can effectively trigger mitochondria-associated

Figure 4  Saponins improve the therapeutic effect of cancer vaccines as adjuvants.
apoptosis and promote the release of HSP70, HSP78, HSP90, CRT, and HMGB1 in melanoma cells. Besides, shikonin also improves the expressions of CD86 and MHC II and finally enhanced tumor immunogenicity to promote the efficacy of shikonin-treated B16 tumor cells- or doxorubicin-treated B16 tumor cells-loaded DCs vaccines. In addition, Lin et al. also reported shikonin would enhance tumor-immunogenicity of tumor vaccines by ICD.

Therefore, the ICD effect induced by natural products not only can enhance the immunogenicity of tumor cells, which makes tumor cells into “therapeutic vaccines”, but also improve the curative effect of DCs-based tumor vaccines. Meanwhile, these natural products also can downregulate the secretion of immunosuppressive factors, such as IL-10, TGF-β, IL-1β, and CCL2, and upregulate the secretion of anti-tumor factors, like IFN-γ and IL12.

### 3.2. Natural products improve the therapeutic effect of cancer vaccines as adjuvants

Most the cancer vaccines, especially peptide-based and gene-based vaccines, have poor immune-stimulate effects and should combine with rational adjuvants to achieve ideal effects. And immunosuppressive factors and cells will also inhibit the therapeutical effect of cancer vaccines. Therefore, the ICD effect induced by natural products not only can enhance the immunogenicity of tumor cells, which makes tumor cells into “therapeutic vaccines”, but also improve the curative effect of DCs-based tumor vaccines. Meanwhile, these natural products also can downregulate the secretion of immunosuppressive factors, such as IL-10, TGF-β, IL-1β, and CCL2, and upregulate the secretion of anti-tumor factors, like IFN-γ and IL12.

#### Table 1

| NCT number   | Natural product | Cancer immunotherapy              | Disease                                   | Status     | Phase |
|--------------|-----------------|-----------------------------------|------------------------------------------|------------|-------|
| NCT03192059  | Curcumin        | Pembrolizumab                     | Cervical cancer/Endometrial cancer/Uterine cancer | Recruiting | II    |
| NCT00470574  | QS-21           | Sialyl lewis-keyhole limpet hemocyanin conjugate vaccine | Breast cancer                           | Completed  |       |
| NCT00036933  | QS-21           | MUC-2-Globo H-KLH conjugate vaccine | Prostate cancer                          | Completed  | I     |
| NCT00049292  | QS-21           | MUC-2-KLH vaccine                 | Prostate cancer                          | Completed  | I     |
| NCT00063632  | QS-21           | MUC1-KLH vaccine                  | Prostate cancer                          | Completed  | I     |
| NCT00033357  | QS-21           | GM2-KLH vaccine                   | Breast cancer                            | Completed  | I     |
| NCT00038189  | QS-21           | TF(c)-KLH conjugate vaccine       | Prostate cancer                          | Completed  | I     |
| NCT0004156   | QS-21           | MUC1-KLH vaccine                  | Breast cancer                            | Completed  | I     |
| NCT00030823  | QS-21           | Globo-H-GM2-Lewis-y-MUC1-32(aa)-Tn(c)-Tn(c)-KLH conjugate vaccine | Breast cancer | Completed |       |
| NCT00006041  | QS-21           | MUC1-KLH vaccine                  | Fallopian tube cancer/ Ovarian cancer/Primary peritoneal cavity cancer | Completed  | I     |
| NCT0006387   | QS-21           | Ras-peptide cancer vaccine        | Colorectal cancer/Pancreatic cancer       | Completed  | I     |
| NCT0001572   | QS-21           | Id-KLH vaccine                    | B cell lymphoma/Follicular lymphoma/Neoplasm | Completed  | I     |
| NCT0004052   | QS-21           | BCR–ABL peptide vaccine           | Leukemia                                 | Completed  | II    |
| NCT0087545   | Saponin-based immunoadjuvant | Polyvalent antigen-KLH conjugate vaccine | Ovarian epithelial cancer/ Fallopian tube cancer/ Peritoneal cancer | Completed  | II    |

Not applicable.
cellular immune responses, especially for cellular immune. And the levels of IFN-γ and IL-4 were significantly enhanced by these saponins to improve the antitumor immunity. Ginsenoside Rg1, the well-known saponins from ginseng, was reported to activate the PBMC-derived DCs through promoting the tumor necrosis factor α (TNF-α), IL-1β, IL-8, and IL-6 [109]. And the mechanism of Rg1 in promoting the activation of DCs was probably through modulating the NF-κB pathway in DCs. This activation is often correlated with the vaccine adjuvants’ action. Wu et al. [101] reported that RelB-/- mice could not develop DC cells due to the genetic defect of NF-κB, which revealed the importance of this pathway for DCs. Then, O’Sullivan et al. [102] indicated the importance of NF-κB activation in T cells by DCs and this function would be enhanced if NF-κB activation in DCs is prolonged. Therefore, the regulation of NF-κB plays a critical role in the induction of mature DCs and that is the key point for the adjuvant activation of Rg1.

Besides, some natural products, which can down-regulate the NF-κB signaling pathway, have also been reported that can sensitize cancer vaccines, but the target issues mainly focus on tumor cells. Kamat et al. [103] found curcumin has the potential to improve the therapeutic outcome of bacillus Calmette-Guerin vaccines, which is the golden standard immune therapy of bladder cancer, through inhibiting NF-κB and TRAIL pathways in cancer cells (Fig. 5A). Lu et al. [104] also reported that curcumin could significantly enhance the effect of the TRP2 peptide vaccine for melanoma. Besides, they found curcumin would decrease the number of immune-suppressive cells (MDSCs and Tregs) in TME by inhibiting the Janus kinase (JAK)-STAT3 signal pathway. In addition, curcumin has been reported to suppress the expression of IDO by blocking the JAK–STAT1 signaling pathway and it sensitizes the FAPαc vaccine for melanoma through this mechanism [105,106]. Wei et al. [107] found the adjuvant effect of low dosages (1 μg/mL) of Dioscorea polysaccharides for DNA vaccines. Their results showed Dioscorea polysaccharides could effectively enhance the anti-tumor effect of DNA vaccines for melanoma through the NF-κB inhibition of tumor cells.

Polysaccharides is the other category of natural products that reportedly sensitize cancer vaccines (Fig. 5B). β-Glucans, the glucose polymers linked together by a 1→3 linear β-glycosidic chain core, is an active compound of polysaccharides for the immune sensitized effect [108]. It could bind to some immune receptors, such as dectin-1, complement receptor 3, and Toll-like receptor (TLR) -2/6, and activate various immune cells. Some polysaccharides, which rich in β-glucans like Dioscorea polysaccharides, have been reported as potential adjuvants for cancer vaccines. Chang et al. [109] evaluated two polysaccharides extracted from Astragalus membranaceus and Codonopsis pilosella for the adjuvant effect of DC-based cancer vaccines for 4T1 mammary carcinoma in mice. They found this combination could significantly enhance the numbers of CD40, CD80, and CD86 markers in DCs [110]. And their data also showed it could up-regulate the secretion of IL-6, TNF-α, and IL-1β. More importantly, this combination would not generate the “cytokine storm”, which is an excessive expression of vaccine adjuvant. Lv et al. [111] investigated the adjuvant effect of basal polysaccharide on DC vaccines for SKOV3 cells. And the results showed that BPS could significantly inhibit the growth of SKOV3 cells and promote the mature of DCs through modulating the expressions of osteopontin, CD44, and matrix metalloprotein-9 (MMP-9). Therefore, it may be a suitable candidate for DC-based vaccines. Mannose and galactose also are two types of polysaccharides that can induce an immune response, but the mechanism is not clear. Ganoderma polysaccharides, which rich in mannose and galactose, can stimulate the maturation of bone marrow dendritic cells (BMDCs) and enhance the secretion of IFN-γ to produce TNF-α. Besides, it also could induce DCs maturation. And Ganoderma polysaccharides-adjuvanted ovalbumin (OVA) immunization promoted the production of specific antibodies and enhanced the OVA-specific T helper type 1 (Th1) cells and cytotoxic T cell (CTL) responses, which could both protect mice from OVA-expressing tumor cells. Their data also showed that the immunization of Ganoderma polysaccharides mainly contribute to engaging pattern-recognition receptors families like TLR4 [112].

TLR4 may be the most important member of the TLR protein for adjuvants of vaccines (Fig. 5C). And it is the only TLR that could activate both MyD88-dependent and -independent (TRIF-dependent) pathways [113]. As one of the TLR4 agonists, monophosphoryl lipid A is approved as a kind of vaccine adjuvant and has shown immunogenic properties without severe side effects. λ-Carrageenan, which is extracted from marine red algae, could effectively enhance E7-specific immune responses induced by E7-peptide vaccines via TLR4 pathway [114], and thus enhance its therapeutic outcomes. In addition, the injection of λ-carrrageenan significantly up-regulated the proportion of M1 macrophages and DCs in TME and it also increased the numbers of activated lymphocytes and Th17 cells in mice spleens [115]. Uncaricin acid C (URC), an active compound from Uncaria hynchophylla, has been demonstrated could activate DCs in a fashion that favors Th1 polarization via the TLR4 pathway, and may be suited for DC-based vaccines [116]. Rutin, extracted from Hedyotis diffusa Willd., could induce cytokines production of immune cells through the TLR4 signaling pathway and enhance the antitumor effect of peptide-based cancer vaccines in HPV-related tumor models [117].

Flavonoid is another type of natural product that reportedly adjuvants potential for cancer vaccines (Fig. 6A). Procyanidin, the biological flavonoid widely distributed in fruit, was evaluated adjuvant effects with B16F10 cancer vaccine for melanoma cancer [118]. The results showed it could significantly enhance T cell-mediated immune responses and promote the secretion of various molecules like perforin, IFN-γ, and TNF-α. They also showed procyanadin could enhance the production of memory T cells induced by cancer vaccines, and then prolonged the survival rate of model rats. Naringenin, one of the most abundant flavonoids in diets, can improve the efficacy of OVA antitumor therapeutic vaccines by increasing intracellular ROS [119]. ROS has been reported to enhance MHC-I antigen cross-presentation through either regulation of the pH in the internalization compartments or antigen oxidation for the MHC-I antigen processing [120,121]. Naringenin-enhanced antigen cross-presentation is dependent on a moderate level of lipid peroxidation that increases antigen leakage from endosomes/lysosomes without impairing DC activation.

Moreover, there are some other types of natural products that have been explored the adjuvants’ potential for cancer vaccines (Fig. 6B). Neem leaf glycoprotein could effectively up-regulate the expression of CD40, CD80, CD83, CD86, and MHCs on the surface of DCs and these matured DCs also could induce a high amount secretion of IFN-γ by Th cells [122]. Besides, neem leaf glycoprotein would help to induce specific immune responses of TSAs through the antigen presentation modulated by macrophage [123]. More importantly, it also could increase the numbers of both central and effector memory CD8+ T cells to generate anti-tumor immunity of cancer vaccines [124]. Carthamus tinctorius could promote the production of IFN-γ and IL-10 of T cells in the spleen and stimulate DCs maturation through the up-regulation of
When DC-based vaccines were injected with antigens and *C. tinctorius*, this combination effectively enhances the numbers of TNF-α and IL-1β and stimulates many immunologic molecules that were mainly expressed on the cell membrane of DCs. In addition, *C. tinctorius* also could increase Teffs production and enhance the therapeutic outcome of cancer vaccines. Antrodia camphorate, a kind of promising adjuvants of human epidermal growth factor receptor-2 (HER-2)/neu DNA vaccines for MBT-2 tumor therapy, could significantly enhance the Th1-like cellular immune responses and then promoted the production of IFN-γ and HER-2/neu-specific Teffs. Therefore, antrodia camphorate could enhance the antitumor effect of the HER-2/neu DNA vaccine.

As mentioned above, saponins, polysaccharides, and flavonoids are three main categories that showed the potential of being qualified adjuvants for cancer vaccines. And the mechanisms of these three categories are different. Saponins mainly rely on its unique chemical structure to punch pores on cell membranes that allow antigens to access into cells. Polysaccharides mainly depend on its compositions that have the immune-stimulating ability. For flavonoid, its effect on cancer vaccines mainly contributes to the unique regulating effect on immune-associated processes, such as ROS and lipid peroxidation. In addition, the NF-κB and TLR4 signaling pathways are two important pathways that are modulated by natural products to sensitize the efficiencies of cancer vaccines.
4. Natural products enhance the therapeutic effect of immune-check points inhibitors

Immune-check point is the other important way for tumor cells to inhibit the Teffs' activities. Seven types of immune-checkpoint antibodies have been applied to clinical anti-tumor therapy. And the PD-1/PD-L1 pathway has stood out among ICTs due to the excellent therapeutic outcomes in many studies and clinical trials. However, the existence of immunosuppressive microenvironments in tumors limits the application of anti-ICTs antibodies. And natural products have been reported to show the ability to regulate the expression of PD-1 and PD-L1 and reverse the immunosuppression.

4.1. Natural products inhibit the expressions of PD-1 and PD-L1 and reverse the immunosuppression

For PD-1 ligand, there are several natural products have been reported to inhibit its expression (Fig. 7A). A kind of triterpenoid saponin isolated from Anemone flaccida inhibited hepatocellular carcinoma (HCC) cell growth by blocking the activation of PD-1 and PD-L1 through the downregulation of STAT3 signaling pathways. And these triterpenoid saponins also could reduce Tregs production and increased the number of splenic immune cells in H22 tumor-bearing mice. Liang et al. investigated the pharmacodynamic of Dendrobium officinale polysaccharides (DOPS) in colorectal cancer, which showed it could effectively improve infiltrating CD8+ cytotoxicity T lymphocyte activity through reducing PD-1 expression and enhance tumor immune response. Ganoderma lucidum polysaccharide combined with paclitaxel (PTX) could reserve the exhausted state of tumor infiltration lymphocytes (TILs) via down-regulating the expressions of PD-1. Besides, G. lucidum polysaccharide also sensitized the therapeutic effect of PTX and restored the gut dyshsiosis induced by PTX. Delphinidin-3-O-glucoside (D3G) and cyanidin-3-O-glucoside (C3G), which active metabolites of anthocyanins (ANC), both can inhibit the expression of PD-1 and PD-L1 in tumor microenvironments of colon cancer cells and induce cancer cell death.

For PD-L1 ligand, its expression is controlled by several pathways, such as JAK-STAT pathway and NF-κB pathway (Fig. 7B and C), and several reviews have summarized the functions of these signaling pathways in regulating the expressions of PD-L1 ligand. Some natural products have been explored the capacity for the down-regulation of PD-L1 through these pathways.

The JAK-STAT signaling pathway is related to the PD-L1 mRNA expression. It has been reported that increasing the activities of JAK and STAT signal transducers will upregulate the expression of PD-L1, and thus the agents that can inhibit the JAK-STAT pathway have the ability to downregulate the PD-L1 expression. Zhang et al. found that berberine, an isoquinoline alkaloid extracted from Coptidis rhizomes, can reduce the expressions of PD-L1 through the JAK–STAT signaling pathway and reverse the resistance induced by doxorubicin in the ovarian cancer cell. Silibinin, a type of flavonoid, has been shown to significantly downregulated PD-L1 expression through the JAK–STAT pathway and increased the immunogenicity of non-small cell lung cancer (NSCLC). Baicalein and its conjugate baicalin, which are active flavonoids of Scutellaria baicalensis Georgi, could downregulate the PD-L1 expression through suppressing STAT3 activity and then reactivate T cells sensitivity to kill tumor cells. A triterpenoid sapogenin monomeric compound extracted from Panax ginseng, panaxadiol reportedly enhanced the activity of CTLs of tumor-cells killing capacity by the suppression of STAT3 activity through JAK1 and JAK2 pathway. And their results also showed panaxadiol downregulated hypoxia-inducible factor-1α (HIF-1α), which also could affect PD-L1 expression. Han et al. investigated tanshinone in hepatocellular carcinoma showed that it can induce apoptosis through the JAK2–STAT3 signaling pathway and simultaneously down-regulate the PD-1 expression caused by TNF-γ, thereby enhancing the immunotherapy effect. Apigenin, a bioavailable flavonoid found in nature, has been reported that could inhibit the high-level expression of PD-L1 induced by IFN-γ through inhibiting phosphorylation of STAT1 in breast cancer cells and melanoma cells.

The NF-κB pathway is the other pathway that can effectively modulate the expression of PD-L1. The mutated or hyperactivated NF-κB can significantly promote the PD-L1 expression. Ginsenoside Rk1, an active compound extracted from ginseng, was reported that could inhibit PD-L1 expression in human lung adenocarcinoma (A549 and PC9) cells through inhibiting the NF-κB pathway. In addition, inhibiting NF-κB transcription also could directly induce the apoptosis of A549 and PC9 cancer cells. Ginsenoside Rg3, the other active compound from ginseng, was identified that could reduce the expression of PD-L1 induced by cisplatin through inhibiting the activation of NF-κB in A549 cells. And it also reported that Rg3 could evaluate the proportion of T cells in NSCLC patients. A novel natural polysaccharide from Cordyceps militaris, CMPB90-1, reportedly restrained the PD-L1-PD-1 axis by downregulating AKT/NF-κB pathway. And then, it also could reverse the inhibitory effects of TAMs on T cells by regulating the secretion of cytokines from M2 macrophages through this pathway. Moreover, Hsu et al.

Figure 6 Natural products improve the therapeutic effect of cancer vaccines as adjuvants. (A) Flavonoids; (B) other natural products.
also found that *C. militaris* could reduce the expression of PD-L1 through modulating the secretions of IFN-γ and TNF-α in oral cancer cells and decrease the secretion of IL-17A. Hesperidin, a flavonoid compound from orange peel, has been reported that could strongly inhibit the mRNA expression and protein activity of PD-L1 through the inhibition of AKT/NF-κB signaling in triple-negative breast cancer. Besides the natural products mentioned above, there are still some natural products that have been shown could regulate PD-L1 expressions. Buzhong Yiqi Decoction (BYD) is a kind of compound preparation made from *Astragalus* (*Radix Astragali*), *Codonopsis* (*Radix Codonopsis Pilosella*), *Trigonol* (*Rhizoma Sparganii*), and other main drugs. Xu et al. found this traditional Chinese decoction could down-regulate the expression of PD-L1 through modulating the secretions of IFN-γ and TNF-α in oral cancer cells and decrease the secretion of IL-17A. Hesperidin, a flavonoid compound from orange peel, has been reported that could strongly inhibit the mRNA expression and protein activity of PD-L1 through the inhibition of AKT/NF-κB signaling in triple-negative breast cancer.

**Figure 7** Natural products down-regulate the expressions of PD-1 and PD-L1.
PD-1 and PD-L1 in gastric cancer cells to promote CTLs activities through the phosphoinositide 3-kinase (PI3K)/AKT/mechanistic target of rapamycin (mTOR) pathway. Besides, this down-regulation effect is further enhanced after the combination with 5-fluorouracil. SA-49, an active compound extracted from Sophora alopecuroides L., was found could induce melanogenesis associated transcription factor by activating protein kinase Cα and subsequently suppressing glycogen synthase kinase 3β, accordingly triggered lysosome-based degradation of PD-L1. Yao et al. found that chaenomeles specioso nakai has the anti-tumor activity of down-regulating the expression of PD-L1 in a dose-dependent manner, thereby inhibiting tumor growth and enhancing its immune activity.

Therefore, it can be found that the mechanism of inhibition of PD-1/PD-L1 by natural products are mainly attributed to the regulation of the related signaling pathways, which have a common modulated effect of ICTs on almost all kinds of tumors. This mechanism can also remodel the immunosuppression in tumors through these signaling pathways, such as MAPK and JNK pathways that can induce immunosuppression and enhance the CAFs proliferation. Xu et al. found that puerarin could significantly inhibit ROS production, which results in the reversing of immunosuppression. The low ROS level also decreases the number of CAFs and this can remove the physical barrier in the 4T1 tumor model, which increases the infiltration of T cells by 2-fold compare to the control group. And the high level of infiltrated T cells enhances the therapeutic outcomes of anti-PD-L1 antibodies.

Although there is no clear conclusion about how to efficiently enhance the therapeutic outcomes of anti-ICTs antibodies, reversing immunosuppression and increasing tumor-infiltrated T cells’ number are two important points that have been commonly accepted. As mention above, the inhibition of COX2 and PGE2 induced by andrographolide and activation of NF-κB in immune cells induced by cryptotanshinone both reverse the immunosuppression in tumor and finally improve the anti-ICTs therapy. In addition, the gut microbiome is also an important factor that modulates responses to anti-PD-L1 therapy. And its mechanism also focuses on the modulation of immunosuppression and enhancement of TpD8* function, and thus the regulation function of intestinal microbiota induced by diosgenin enhances the outcome of anti-PD-L1 therapy. For increasing the infiltrated T cells in tumors, Xu et al. found that puerarin could achieve it through downregulation of CAFs, which open the physical barrier of these T cells. In addition, the low ROS level induced by puerarin also reversing the immunosuppression in the tumor. Therefore, natural products have their unique advantages in the combination of anti-ICTs therapy.

5. Natural products sensitize the adoptive cell immunotherapy

Adoptive cell immunotherapy, especially T-cell-based adoptive immunotherapy, is a promising anti-cancer immunotherapy and has shown excellent therapeutic outcomes in the patients. Briefly, the process of this immunotherapy consists of three steps: 1) tumor fragments extracted from patient are cultured in vitro under the reaction of IL-2; 2) lymphocytes are overgrown under this condition and tumor cells will be killed after 2–3 weeks; 3) the pure lymphocytes are expanded to about 10^11 cells and then adoptive back into patients. Therefore, the number and activity of the target lymphocytes cultured in vitro is the key point for adoptive cell immunotherapy. However, this approach remains challenging due to a series of reasons, including...
the restriction of antitumor effect by immunosuppressive mechanism, the insufficient antitumor effect of T-cells cultured in vitro, and expensive therapeutic cost. As the in-depth investigation of the immune-mediated function of natural products, these obstacles reportedly could be conquered by some natural agents.

First of all, natural products can efficiently expand the target lymphocytes in vitro and it also can keep the activities of these cells. Ma et al.\(^{168}\) reported the therapeutic effect of \(H.\) \(diffusa\) polysaccharides (HDP) on the adoptive treatment of cytokine-induced killer cells (CIKs), which is a kind of safety and feasibility adoptive cells therapy in anti-tumor treatment (Fig. 9B). Their results showed that HDP could up-regulate the percentage of CD3\(^+\) and CD56\(^+\) CIK cells. And it also had great anti-tumor ability due to the promotion of IFN-\(\gamma\) and TNF-\(\alpha\) productions. Besides, there is a lower ratio of apoptosis in CIK cells treated by HDP. Ju et al.\(^{169}\) proved that 6-gingerol, the major active compound of ginger (\(Zingiber officinale\)), could effectively enhance the Teffs production in tumors (Fig. 9C). After the treatment with 3.0 \(\mu\)g/mL of 6-gingerol, they found it could effectively inhibit tumor growth due to the increased number of Teffs. Then, they inject CD8\(^+\) T cells, which were purified from the 6-gingerol pretreated tumor-bearing mice, into EG7 tumor-bearing mice. And they found more CD8\(^+\) T-cells infiltrated in tumors and more cells undergoing division in the 6-gingerol-treated group compared to the control group. Moreover, they treated CD8 T cells with 6-gingerol in vitro and transferred them into EGE7 tumor-bearing RAG2\(^{-/-}\)/C0 mice. And these 6-gingerol-treated CD8 T-cells also showed outstanding anti-tumor activity. These results showed the great potential of natural products in adoptive cell therapy for cell expansion in vitro and this may reduce the cost of this immunotherapy.

Then, natural products also can enhance the therapeutic outcomes of adoptive cell therapy by improving the Teffs' activities.
enhancing the infiltration of lymphocytes, and remodeling tumor immunosuppressive microenvironments. Alatrash et al.\textsuperscript{170} reported fucosylation could enhance the homing of antigen-specific CTLs to malignant niches and increase its anti-tumor activity (Fig. 9D). Besides, fucosylation would not increase the homing of CTL in normal tissues. Their results showed fucosylation could alter CTL trafficking, cytolytic machinery, synapse formation, and expression of distinct activating surface molecules. And their method could effectively enhance the therapeutic effect of T-cells-based adoptive immunotherapy. Banerjee et al.\textsuperscript{171} found that neem leaf glycoprotein (NLGP) could significantly restrict the growth of melanoma through vascular normalization by regulating cell-mediated immunity to enhance the infiltration of immune cells (Fig. 9E). Their results showed the adoptive splenic immune cells therapy treated Figure 9 Natural products enhance the therapeutic effect of adoptive cell transfer therapy. (A) The generation of anti-tumor immune cells used for adoptive cell therapy. Reprinted with the permission from Ref. \textsuperscript{170}. Copyright © 2008, nature publishing group. (B) \textit{Hedyotis diffusa} polysaccharides improve the efficiency of adoptive treatment of cytokine-induced killer (CIK) cells; (C) 6-gingeral expand the number of T cells in vitro for adoptive therapy; (D) neem leaf glycoprotein (NLGP) can significantly enhance the activity of immune cells in spleen; (E) Fucosylation can enhance the anti-tumor activity of T cells of adoptive therapy; (F) Curcumin improve the efficiency of adoptive T cells treatment.
by NLGP could effectively normalize the angiogenesis, which is mainly induced by tumor-associated microenvironments. Besides, they also analyzed the expression of VEGF, VEGFR2, and CD31 in adoptive transfer immune cells pretreated by NLGP and their expressions were all down-regulated. Thus, NLGP may be suitable sensitize for adoptive immune cells therapy. In addition, Chang et al.\textsuperscript{172} investigated that curcumin could enhance adoptive T cells therapy through reversing the immunosuppression in tumors (Fig. 9F). They found that the curcumin could enhance the activity of T\textsubscript{effs}, which could alter the tumor immunosuppression environment. And curcumin could increase the accumulation and function of T-cells by blocking various immunosuppressive factors and cells, including TGF-\textbeta, indoleamine-pyrrole 2,3-dioxygenase (IDO), and Tregs. In addition, the treatment of curcumin also would affect granzyme B promoter-conjugated optical reporter to improve the cytotoxicity of Teffs. And these results suggested that adoptive therapy with curcumin, a kind of multitargeting drug, has the potential for clinical application.

As mention above, natural products not only can expand the target immune cells \textit{in vitro}, but also enhance the therapeutic effects of this immunotherapy, which shows great potential in adoptive immune-cells therapy.

6. Nano-drug delivery system enhance the therapeutic outcomes of natural products in cancer immunotherapies

After so many advantages have been explored in natural products in cancer immunotherapies, the shortages of natural products were also been emphasized in these studies and limited its further application, including low solubility, low bioavailability, and low tumor targeting. Because of these limitations, most the natural products have to apply to patients with a huge dosage, which may increase the risk of inducing side effects. Therefore, advanced drug-delivery systems should be designed for natural products to overcome these limitations. And the nano-drug delivery system, such as liposomes, micelles, and nano-particles, stands out due to the better outcomes of tumor-targeting, anti-tumor activities, immune-sensitization, stability, and safety.

Xiao et al.\textsuperscript{173} reported a novel nanoparticle-linked anti-PD-1 antibodies on its surface and encapsulating curcumin. And this kind of nanoparticle was designed with dual pH sensitivity (Fig. 10A). Compare to curcumin solution, this kind of nanoparticles enhances the cellular uptake of curcumin. Besides the enhanced permeability and retention effect (EPR) effect, the decoration of anti-PD-1 antibodies will also help nanoparticles binding to PD-1\textsuperscript{+} T cells. Owing to the pH sensitivity, these nanoparticles showed higher targeting for tumor cells. Then, more curcumin was delivered into the tumor area to inhibit the NF-kB pathway, which significantly inhibits the production of CCL-22, TGF-\textbeta, and IL-10. And it will decrease the number of T\textsubscript{reg} cells and enhance the activities of T\textsubscript{effs}. This nanoparticle effectively co-delivery anti-PD-1 antibodies and curcumin into tumors and showed excellent therapeutic outcomes \textit{in vivo} and \textit{in vitro}.

Zhang et al.\textsuperscript{79} designed a novel nanoparticle encapsulating quercetin (Q) and alantolactone (A), which promoted antitumor responses through the ICD effect for microsatellite-stable
Table 2  List of natural products that improve cancer immunotherapy, including cancer vaccines, immune-check points inhibitors and adoptive cell transfer therapy.

| Category      | Source                | Natural product                  | Key points of sensitizing immunotherapy                                                                 | Ref.   |
|---------------|-----------------------|----------------------------------|--------------------------------------------------------------------------------------------------------|--------|
| Saponins      | Ginseng               | Ginsenoside Rg3                  | • Trigger “ICD” effect  
• Down-regulate the expression of PD-L1  
• Enhance the anti-cancer effect of cancer DC-based vaccines through NF-κB pathway | 74,141,142 |
|               |                       | Ginsenoside Rg1                  | • Enhance the anti-cancer effect of cancer DC-based vaccines through NF-κB pathway                      | 97     |
|               |                       | Ginsenoside Rk1                  | • Down-regulate the expression of PD-L1 through NF-κB pathway                                           | 140    |
| Astragalus    | Astragalus saponins   |                                  | • Down-regulate the expression of PD-L1 and PD-L1 through STAT3 pathway                                 |        |
|               |                       |                                  | • Enhance the anti-cancer effect of cancer protein-based vaccines                                       |         |
|               |                       |                                  | • Down-regulate the expression of PD-L1 and PD-L1 through STAT3 pathway                                 |         |
| Polysaccharides | Dioscorea             | Dioscorea polysaccharides        | • Down-regulate the expression of PD-L1 and PD-L1 through STAT3 pathway                                 | 104    |
|               |                       |                                  | • Enhance the anti-cancer effect of cancer DNA vaccines                                               |         |
|               |                       | Astraagulus membranaceus         | • Enhance the anti-cancer effect of cancer DC-based vaccines with Codonopsis polysaccharide           | 106    |
|               |                       | Codonopsis polysaccharides       | • Enhance the anti-cancer effect of cancer DC-based vaccines with Astraagulus polysaccharide          | 106    |
|               |                       | Basil                            | • Enhance the anti-cancer effect of cancer DC-based vaccines                                           | 108    |
|               |                       | Gallodema polysaccharides        | • Down-regulate the expression of PD-L1 and PD-L1 through STAT3 pathway                                 | 109    |
|               |                       |                                  | • Enhance the anti-cancer effect of cancer vaccines                                                  |         |
|               |                       | Dendrobia officinae              | • Down-regulate the expression of PD-L1 and PD-L1 through STAT3 pathway                                 | 126    |
|               |                       | Dendrobia officinae polysaccharides | • Down-regulate the expression of PD-L1 through NF-κB pathway                                         | 143    |
|               |                       | Codonopsis polysaccharides       | • Enhance the anti-cancer effect of adoptive CIK cells immunotherapy                                 | 157    |
|               |                       | Basil                            | • Down-regulate the expression of PD-L1 and PD-L1 through NF-κB pathway                                 |         |
|               |                       | Gallodema polysaccharides        | • Enhance the anti-cancer effect of cancer DC-based vaccines                                           |         |
| Flavonoids    | Onion                 | Quercetin                        | • Down-regulate the expression of PD-L1 and PD-L1 through STAT3 pathway                                 | 126    |
|               | H. Diffusa Wild       | Rutin                            | • Trigger “ICD” effect (with alantolactone at 1:4 ratio)                                               | 76     |
|               |                       | Procyanidin                      | • Enhance the anti-cancer effect of cancer peptide-based vaccines                                      | 115    |
|               | Fruits                | Naringenin                       | • Enhance the anti-cancer effect of cancer vaccines                                                  | 116    |
|               | Pomelo                | Silibinin                        | • Down-regulate the expression of PD-L1 through JAK-STAT pathway                                     | 134    |
|               | Silybum marianum (L.) Gaertn. |                       | • Down-regulate the expression of PD-L1 through JAK-STAT pathway                                     | 135    |
|               | Scutellaria baicalensis | Baicalein, Baicalin              | • Down-regulate the expression of PD-L1 through JAK-STAT pathway                                     | 138    |
|               | Celery                | Apigenin                         | • Down-regulate the expression of PD-L1 through JAK-STAT pathway                                     | 145    |
|               | Orange peel           | Hesperidin                       | • Down-regulate the expression of PD-L1 through NF-κB pathway                                         | 145    |
|               | Kudzu root            | Puerarin                         | • Enhance the therapeutic outcome of anti-PD-L1 antibody through regulation of CAFs                  | 165    |
| Others        | Chili pepper          | Capsaicin                        | • Trigger “ICD” effect                                                                                 | 68     |
|               | Grapes                | Resveratrol                      | • Trigger “ICD” effect                                                                                 | 75     |
|               | Ginger                | Curcumin                         | • Enhance the anti-cancer effect of cancer peptide-based vaccines                                      | 101,158|
|               | 6-Gingerol            |                                  | • Enhance the anti-cancer effect of adoptive T cells immunotherapy                                   | 161    |
Table 2 (continued)

| Category       | Source                             | Natural product                        | Key points of sensitizing immunotherapy                                                                 | Ref.  |
|----------------|------------------------------------|----------------------------------------|--------------------------------------------------------------------------------------------------------|-------|
| Inula racemosa | Lithospermum erythrorhizon         | Alantolactone, Shikonin                | ● Trigger “ICD” effect (with quercetin at 1:4 ratio)                                                   | 76    |
|                | Marine red algae                   | λ-Carrageenan                          | ● Enhance the anti-cancer effect of cancer peptide-based vaccines                                       | 79    |
|                | Anthocyanin                        | Dephinidin-3-O-glucoside (DSG)         | ● Down-regulate the expression of PD-1 and PD-L1 through STAT3 pathway                                | 166   |
| Salvia miltiorrhiza | Tanshinone                      | Crytotsinholone                         | ● Down-regulate the expression of PD-L1 through JAK–STAT pathway                                      | 128   |
| Sophora alopecuroides | L. Andrographis paniculata        | SA-49, Andrographolide                 | ● Enhance the anti-cancer effect of anti-PD-L1 antibody (clone 10F9G2)                                | 133   |
| Seaweed        | Neem leaf                          | Fucose, Neem leaf glycoprotein         | ● Enhance the anti-cancer effect of adoptive T cells immunotherapy                                     | 152   |
|                |                                    |                                        | ● Enhance the anti-cancer effect of adoptive cells immunotherapy                                      | 147   |
|                |                                    |                                        | ● Down-regulate the expression of PD-L1 through JAK–STAT pathway                                      | 149   |
|                |                                    |                                        | ● Enhance the anti-cancer effect of adoptive cells immunotherapy                                     | 159   |
|                |                                    |                                        | ● Enhance the anti-cancer effect of adoptive cells immunotherapy                                     | 160   |

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colorectal cancer (Fig. 10B). They found the synergistic effect of quercetin and alantolactone at ICD effect and the anti-tumor activities were maximized at the molar ratio of 1:4. To deliver this effective molar ratio into the target area, they developed a new micelle encapsulated with Q and A, which was prepared with DSPE-PEG2000 and δ-α-tocopherol polyethylene glycol succinate (TPGS) through the ethanol injection method. And they also found the drug release ratio of these Q-A micelles was also approximately 1:4, which is the optimal molar ratio. After various treatments of these Q-A micelles, a dramatic increase of CRT↑ cells induced by the ICD effect and a significant decrease of Tregs and MDSCs also were reported. Moreover, the tumor-promoting inflammation was also inhibited by the G-M micelles treatment.

Yu et al.172 developed a kind of gold nanoparticle (Au-NP) containing Ganoderma lucidum polysaccharide (GLP) to overcome the drawbacks of GLP solutions, such as the short half-life, instability, and low tumor targeting (Fig. 10C). And the results also showed the good characteristics of the GLP-AuNP including increased instability, enhanced tumor targeting, and long-circulating time. Meanwhile, these GLP-AuNPs also kept the immunomodulated function of GLP like inducing dendritic cell (DC) activation and promoting the production of Teffs in the spleen. Besides, GLP-AuNPs exhibited strong inhibitory effects on 4T1 tumor growth and pulmonary metastasis when combined with doxorubicin. This work suggests that polysaccharides from natural herbs can be incorporated into nanocomposites with immunoregulatory characteristics for enhanced efficacy in tumor therapy.

Gu et al.173 successfully encapsulated the immunopotentiator Angelica Sinensis polysaccharide (ASP) and OVA into poly(lactic-co-glycolic acid, PLGA) to formulate the novel NPs-based vaccine delivery system (Fig. 10D). This kind of NPs showed good stability at 4 °C. And the OVA in the core of NPs could effectively release at 37 °C. Besides, these NPs also could enhance the cellular immune response through improve the activity and proliferation of Teffs. Furthermore, a strong specific antibody response induced by OVA and ASP is observed in this study. All of these results proved that the NPs vaccine delivery system could induce longer immune responses compared to the traditional vaccine preparations.

The Liposome is another vesicle to deliver antigen protein into the target area. Zhang et al.99 developed a liposome delivery system of A. saponins and basic fibroblast growth factor (bFGF) antigen to maximize the anti-tumor immune responses induced by these antigens. Through the adjuvant function of A. saponins and bFGF antigen protein, this liposome delivery system could induce lots of specific antibodies and enhance the production of IFN-γ in BALB/c mice. They also found this system could effectively inhibit the angiogenesis in TME, which is an additional anti-tumor function of this novel-designed liposome.

Lu et al.104 reported a kind of curcumin micelles that can remodel the suppressive immune microenvironment to improve the activity of a lipid-based the TRP2 peptide vaccine (Fig. 10E). The combination of curcumin-polyethylene glycol conjugate (CUR-PEG) micelles and TRP2 peptide vaccine showed an outstanding therapeutic effect compared to individual treatments. Moreover, it significantly boosted cytotoxic T-lymphocyte responses and IFN-γ secretion. Besides, this combination therapy also inhibited the production of immunosuppressive factors (such as IL-6 and chemokine ligand 2) and enhanced the number of proinflammatory cytokines (such as TNF-α and IFN-γ). A phenotype switch of M2 to M1 of this kind of micelles has also been reported in this study. These results all showed the great potential of CUR-PEG micelle as a novel adjuvant for peptide cancer vaccines.

Above all, there are some unique advantages of nano-drug delivery systems about improving the therapeutic effect of natural products. First of all, it can efficiently keep the activities and sensitized effect of cancer therapy and increase the circulation time of natural products. Then, it also enhances the tumor-
targeting through the modification of some tumor-specific species and decreases the dosage of natural products. Thirdly, the nano-sized particles of natural products reported by previous studies also showed good stability and safety. Therefore, the nano-drug delivery system is an ideal strategy for natural products.

7. Summary and conclusions

After concluding and investigating the previous studies about natural products sensitizing cancer immunotherapy, we find that natural products can effectively enhance the therapeutic effects of all these immunotherapies, including cancer vaccines, immunomodulatory agents, and adoptive cells immunotherapy. In addition, natural products successfully expand the indications of immunotherapy into “cold tumor”, such as triple-negative breast tumor, Lewis lung tumor, colorectal tumor model, and so on.

As the review is shown, natural products can enhance the specific cancer immunotherapy through modification of the corresponding mechanisms, such as the induction of ICD effect for cancer vaccines, the increase of infiltrated T cells for anti-ICD antibodies, and the expansion of the target immune cells in vitro for adoptive immune-cells transfer therapy. More importantly, as for the immunosuppressive factors and cells in tumors, which is also the key element for passivating all kinds of cancer immunotherapies, natural products also have shown outstanding effects on it, such as inhibition of secretion of immunosuppressive factors (TGF-β, PGE2, IL-10 and so on), promotion of secretion of anti-tumor immune factors (IFN-γ, TNF-α, IL-1β and so on), downregulation of the number of immunosuppressive cells (Tregs, MDSCs, M1 macrophage cells, and so on) and enhancement of the activities and number of Tcells. All of these effects of natural products can contribute to the reversion of tumor-associated immunosuppressive microenvironment.

According to Table 2, saponins, polysaccharides, and flavonoids are three main categories that can improve immunotherapy. Some of them even could sensitize two or more kinds of immunotherapy, such as ginsenoside Rg3, ganoderma polysaccharides, and curcumin. And the mechanism mainly depends on the regulation of associated signaling pathways. Some pathways even can improve the therapeutic effects of different immunotherapies, such as the NF-κB pathway for cancer vaccines and anti-PD-L1 antibodies. The reason for that mainly contributes to the connection between these pathways and immunosuppression with tumors. As mentioned in part 1, tumor cells can “hijack” (upregulate or downregulate) some signaling pathways, which associate with the immune system, to induce immunosuppression. Therefore, the agents that can regulate these pathways to a normal level will reverse the immunosuppression and sensitize cancer immunotherapy. Natural products are such agents. And this is also their biggest advantage compared to other agents. According to this unique mechanism, as mentioned above, some natural products even can sensitize two or more kinds of cancer immunotherapies.

Besides the significant sensitized effect of natural products into immunotherapy, there are many advantages of application natural products as sensitizers compared to the investigation of new agents. Firstly, most of them mentioned in this review have been approved by the FDA, the cost of increasing indications to them is much lower than developing whole new agents. Then, most the natural products have wide treatment windows and high security. More important, natural products still have other functions like anti-viral, anti-bacterial, anti-inflammatory, anti-fungal, and anti-cancer properties. And these functions could further help the anti-tumor treatments.

Moreover, there are several studies that investigated how to use nanotechnology to overcome the shortages of natural products, such as low solubility, low bioavailability, and low tumor targeting. And these new nanoparticles of natural products not only showed higher tumor targeting and long-term circulation, but also further enhance the anti-tumor effect of immunotherapy. Nanotechnology promotes natural products to be the better sensitizer of cancer immunotherapy.

However, there are still some problems that should be solved to make natural products apply to cancer immunotherapy better. First of all, as for so many natural products combined with cancer immunotherapies, it should be screened for the most suitable one based on the specific cancer immunotherapy. For example, for peptide vaccines or mRNA vaccines with poor immune-stimulate effects, the suitable natural products should have the ability to improve this aspect, such as enhancing the antigen-presenting process, but for anti-ICD therapy, the effect of increasing T infiltrated cells’ number is the most important character for the suitable natural products. Secondly, the signaling pathways related to the immune system and cancer immunotherapy should be explored more deeply and comprehensively. It can be found that the sensitization of natural products on cancer immunotherapy mainly depends on the specific signaling pathways associated with the immune system, and thus a comprehensive understanding of these signaling pathways will be a beneficial to selecting the more effective natural products. Thirdly, there should be more data to provide information about the function of natural products in cancer immunotherapy. Although various natural products that can sensitize cancer immunotherapy have been discussed in this review and some of them even have been applied into clinical trials, the data of this field is still too little compare to that of natural products used in chemotherapy and radiotherapy.

Above all, this review collects and concludes the emerging studies about natural products sensitizing cancer immunotherapies. And there is a clear conclusion that natural products have positive effects on cancer immunotherapies, which mainly depends on their unique modification of tumor immunosuppressive microenvironment. The emerging role of natural products in cancer immunotherapy still has more possible and is worthy to be paid more attention.

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Author contributions

Songtao Dong: conceptualization, collecting references and writing; Xiangnan Guo: collecting references; Fei Han: conceptualization, methodology, writing—review & editing; Zhonggui He: conceptualization, supervision, project administration; Yongjun Wang: conceptualization, methodology, writing—review & editing, supervision, project administration, funding acquisition.

Conflicts of interest

The authors declare no conflicts of interest.
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