Med. Sci. **2014**, *2*, 1-22; doi:10.3390/medsci2010001

OPEN ACCESS

medical sciences

ISSN 2076-3271

www.mdpi.com/journal/medsci

Review

Antitumor Immunity and Dietary Compounds

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Received: 15 November 2013; in revised form: 16 December 2013 / Accepted: 16 December 2013 / Published: 27 December 2013

Abstract: The mechanisms by which natural dietary compounds exert their antitumor effects have been the focus of a large number of research efforts in recent years. Induction of apoptosis by inhibition of cell proliferative pathways is one of the common means of cell death employed by these dietary compounds. However, agents that can activate an antitumor immune response in addition to a chemotherapeutic effect may be useful adjuvants or alternative therapies for the treatment of cancer. The focus of this review is to highlight representative dietary compounds, namely *Withania somnifera*, *Panax ginseng*, curcumin and resveratrol with special emphasis on their antitumor immune mechanism of action. Each of these dietary compounds and their sources has a history of safe human use as food or in herbal medicine traditions, potentially making them ideal therapeutics. Here we report the recent advances in the cellular immune mechanisms utilized by these compounds to induce antitumor immunity. Taken together, these findings provide a new perspective for exploiting novel dietary compounds as chemoimmunotherapeutic anti-cancer agents.

Keywords: antitumor immunity; natural products; immunomodulation; cancer; *Withania somnifera*; *Panax ginseng*; curcumin; resveratrol

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1. Introduction

In 1909, Paul Erlich predicted that cancer would occur at "incredible frequency" if host defenses did not prevent the outgrowth of continuously arising cancer cells [1]. This idea became the basis for the field of tumor immunology and the generation of the cancer immunosurveillance hypothesis by Burnet and Thomas. The cancer immunosurveillance hypothesis suggests that constant occurrence of cancer is controlled by the host's defense mechanisms [2,3]. Increased incidence of non-viral tumors in immunosuppressed organ transplant patients [4] and studies showing that cancer patients develop spontaneous immune responses to their tumors [5] (e.g., Her-2neu breast cancer [6]) provide correlative evidence supporting the existence of cancer immunosurveillance in humans.

One of the most important aspects of tumor immunotherapy and the generation of an effective antitumor response is the requirement of tumors expressing tumor specific antigens that can be recognized by cells of the adaptive immune system: $CD4^+$ and $CD8^+$ T-cells and/or B-cells [7]. Stimulation of these lymphocytes can occur from the tumor itself but typically involves professional antigen presenting cells (APCs) primarily dendritic cells (DCs). Tumor cells secrete or present danger signals on the plasma membrane that facilitates engulfment by APC and initiates cross presentation, a process involving phagocytosis of cancer cells and subsequent presentation of tumor antigens to cognate T-cells [8,9]. This process also induces the secretion of pro-inflammatory cytokines from both DCs and activated T-cells including IFNγ, IFNα, IFNβ, IL-12, TNFα and IL-2.

The profile of cytokines expressed by T cells during an immune response defines whether a T helper 1(Th1) or T helper 2 (Th2) response is activated. IFNγ is the predominant Th1 cytokine that plays a key role in mediating pro-inflammatory responses. Cytokines such as IL-4 serve as anti-inflammatory agents and can dampen an endogenous antitumor immune response. Th1 responses are typically cell mediated and have been demonstrated to be more effective for antitumor immunity than the Th2 response, which activates humoral immunity. Cancer patients often exhibit decreased expression of Th1 cytokines and enhanced Th2 cytokines systemically as well as within the tumor. One of the goals of antitumor immunotherapy is therefore to interfere with the Th2 skew observed in cancer patients and induce a predominantly antitumor Th1 response.

Cytokines function as the mediators of an immune response however, licensed CD8 cytotoxic T-lymphocytes (CTLs) are the effector cells that recognize tumor specific antigens and exert potent antitumor cytotoxic activity on cancer cells via the pore forming molecule perforin [10]. Perforin forms holes in the membrane of target cells allowing for the secretion of the cell death effector protein Granzyme B by CTLs which facilitates tumor killing.

CD4 T helper cells play a role in generating a potent antitumor response and predominantly induce their cytotoxic activity by upregulating Fas ligand on the cell surface within several hours after T-cell activation [11]. Fas ligand interaction with Fas, a TNF family of receptors, on target cells activates caspases that initiate DNA fragmentation and ultimate apoptosis of target cells [11]. CD4 T cells can also activate effector functions in other immune cell types [12], as B-cells can become activated to secrete antitumor specific antibodies via CD4 T cell help [13,14]. These antibodies primarily exert antitumor effect by antibody dependent cellular cytotoxicity (ADCC) mechanisms as translated in trastuzumab for Her-2/neu breast cancer [15] and rituximab for $CD20^+$ lymphomas [16]. Furthermore, activated B cells can operate as efficient APC and optimize the expansion of tumor antigen specific

CD4 and CD8 T cells [17]. The direct antitumor effect of B cells has been proven by enhanced tumor development in the absence of B cells in a melanoma model [18].

Another immune cell type capable of inducing potent antitumor cytotoxicity is the Natural Killer (NK) cell. Unlike CD8 T cells, NK cells are members of the innate immune system and lack the antigen specificity that is unique to adaptive immune cell types. They however, exhibit similar cytotoxic activity to CD8 T cells when stimulated with pro-inflammatory cytokines such as IL-12, IL-15 and IL-18. Upon activation, NK cells produce further cytotoxic pro-inflammatory cytokines IFNγ, GM-CSF and TNFα as well as induce killing of tumor cells by release of perforin and Granzyme B [19,20]. The inflammatory cytokines secreted by NK cells present in the tumor microenvironment (TME) can cause up-regulation of MHC Class I on tumor cells thus maximizing the antitumor response by allowing increased expression of tumor antigens to CD8 T cells [21]. NK cells can also kill target tumor cells by ADCC through the interaction of FcγRIII (CD16) receptor expressed on NK cells which recognizes tumor cells that are coated with IgG molecules [20].

In order to effectively eradicate growing tumor cells, effector lymphocytes must traffic to the tumor microenvironment (TME) where they can execute their effector functions. The TME is however a highly immunosuppressive environment and effector cells must overcome several mechanisms of immune suppression in order to effectively handle the growing tumor [22,23]. Tumor cells readily down-regulate expression of the MHC Class I molecule thereby decreasing tumor surveillance by activated T-cells [24]. Inhibitory molecules such as PD-L1 and PD-L2 on the surface of tumor cells bind to receptors on activated T-cells (PD-1) causing T-cell anergy or exhaustion [25,26]. Paracrine immunosuppressive mediators secreted by tumor cells such as prostaglandin E2 (PGE-2), adenosine, TGFβ, VEGF-A and indoleamine 2,3-dioxygenase (IDO) inhibits T-cell function and adhesion to tumor endothelium as well as suppress dendritic cell maturation and activity [27]. Finally the TME is characterized by the recruitment of immunosuppressive immune cells such as T regulatory cells (Tregs) [28] and myeloid derived suppressor cells (MDSCSs) that suppresses antitumor immunity [29].

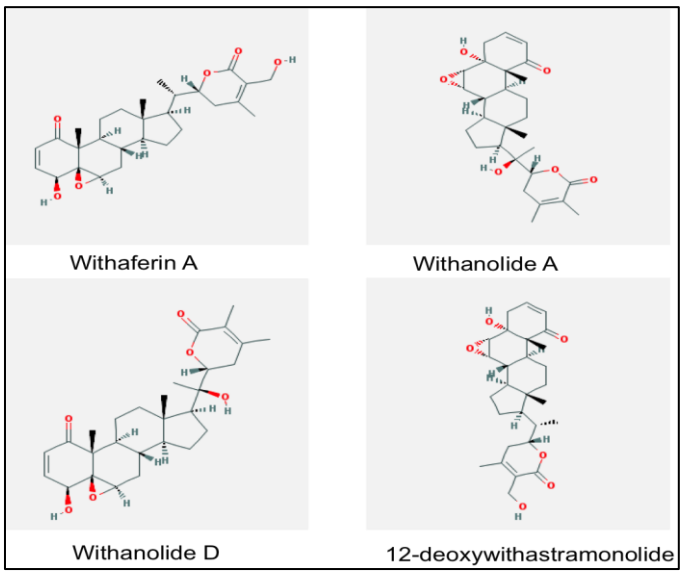
The goal of immunotherapy is thus to engage effective immunity against tumor associated antigens and specifically target mechanisms of immune suppression induced by the tumor. A successful immunotherapeutic strategy must generate potent effective antitumor immune responses and exhibit low toxicity to immune cell types and peripheral normal cells and tissues. In this review we draw special attention to four highly studied natural dietary compounds namely *Withania somnifera*, *Panax ginseng*, curcumin and resveratrol that are known anti-cancer agents. We explore recent research findings on the antitumor immune mechanisms employed by these compounds as well as the current knowledge on their safety and toxicity profiles.

2. *Withania somnifera*

Withania somnifera (WS) also known as Ashwaghandha and commonly called “Indian Winter Cherry” or “Indian Ginseng” has been part of traditional Ayurvedic medicine for many centuries. Formulations of WS from the root or leaves of the plant have traditionally been used as a general tonic to increase longevity and vitality [30]. WS has been shown to be effective against arthritis, rheumatism, cancer and mental disorders such as schizophrenia [31–33]. Over 35 chemical constituents of WS have been isolated and classified, of which the steroidal lactones (Withanolides and

Withaferins) are the most biologically active components (Figure 1) [30,34–37]. Withaferin A (WA) is the most widely studied steroidal lactone for its anti-cancer properties [38] and Withanolide A is the component that is responsible for the immunomodulatory activity of WS [39,40].

Figure 1. Steroidal lactones of *Withania somnifera.* Chemical structures were taken from the PubChem Substance and Compound database (pubchem.ncbi.nlm.nih.gov). The unique chemical structure identifiers are: 265237 (Withaferin A-Depositor: Chembase.cn) [34], 11294368 (Withanolide A-Depositor: Chembase.cn) [35], 161671 (Withanolide D-Depositor: ChEBI) [36], 44576309 (12-deoxywithastramonolide-Depositor: Chembase.cn) [36].



Withanolide A can modulate various effector functions when administered to Balb/c mice by polarization of T helper type 1 (Th1) responses while decreasing T helper type 2 (Th2) axis [40]. Up-regulation of Th1 responses such as pro-inflammatory cytokines by Withanolide A was directly related to its antitumor function as sera from tumor bearing mice treated with WS showed increased secretion of IFNγ and IL-2 [41]. IL-4 was undetectable in the serum of WS treated mice suggesting that WS suppresses the Th2 immune response while simultaneously up-regulating the antitumor Th1 response. This can be correlated with a significant enhancement in the proliferative activity of both CD4 and CD8 T cells in the serum of tumor bearing mice treated with 200 mg/kg WS [41]. T and B-cell proliferation was significantly induced in splenocytes isolated from WS treated mice with or without stimulation with the mitogens Concavalin A (Con A) and lipopolysaccharide (LPS) respectively [42]. WS also increases the potency of other immune cell types such as NK cells and APC that may relate to its antitumor immunity. Direct administration of WA increased NK cell population by 20% [41] and an increase of 49% in its cytotoxic activity by ADCC which was induced two days earlier compared to untreated mice [42]. APCs isolated from blood samples of tumor bearing mice had increased expression of maturation and co-stimulation markers CD80 and CD40 and CD40L on T-cells [41] indicating that WS plays a role in DCs mediated activation of T-cells.

In 1971, Shohat and Joshua conducted the only published study to date on the antitumor immune action of the primary anti-cancer component of WS, Withaferin A [43]. Using an Ehrlich Ascites carcinoma model in Swiss mice they determined that WA induces complete tumor rejection and

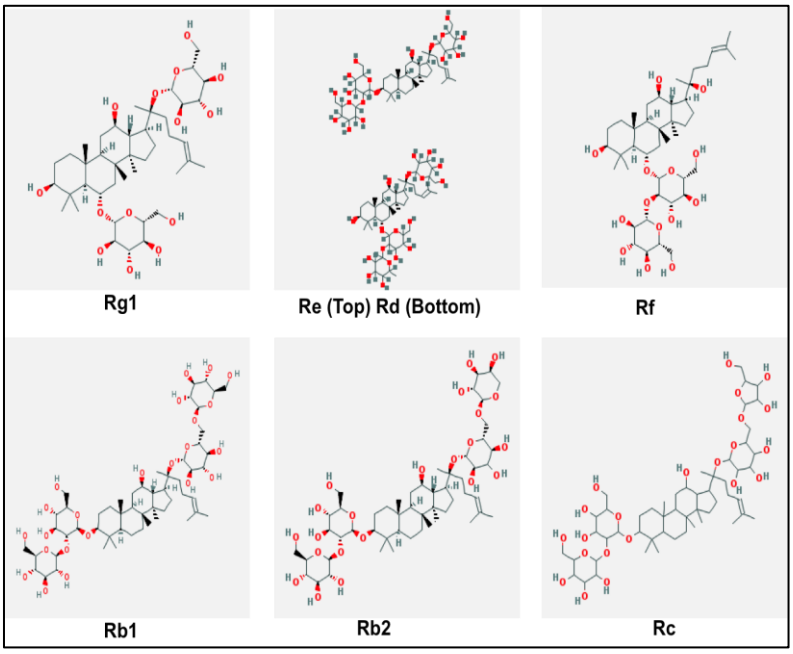
protection from re-challenge indicating the generation of immunological memory against the tumor. This was, however, short-lived as immune protection lasted only two months, post initial tumor challenge. The mechanism of immune rejection was primarily macrophage mediated as there was an increase in peritoneal macrophages and transfer of these macrophages from cured mice was able to induce tumor rejection. Our laboratory is studying how WA affects antitumor immunity in a murine model of breast cancer. Our unpublished data [44] demonstrates that WA induces immunogenic cell death in cancer cells as defined by the expression of heat shock proteins (HSPs) such as HSP70, HSP90 and calreticulin on the plasma membrane of tumor cells. Each of these ICD mediators binds to receptors on DCs resulting in activation and maturation of dendritic cells and the secretion of inflammatory cytokine IL-12 [45]. We also show that WA treated tumor cells can protect mice against live tumor challenge further underscoring the immunogenic nature of WA induced cell death on tumor cells. The ability of WA to overcome immune suppressive factors in the TME was recently explored and it was observed that WA inhibits the function of the tumor inhibitory immune cell type MDSCS [46]. WA suppressed the ability of MDSCS to secrete reactive oxygen species (ROS), which is a key mechanism of MDSCS suppressive activity on T-cells. Taken together, the current findings indicate that *Withania somnifera* and its constituents are effective antitumor immunotherapeutic molecules that positively affect all aspects of immune modulation of cancer growth.

3. *Panax ginseng*

Panax ginseng (*P. ginseng*) has historically been used as a part of traditional medicine in Korea and China for over 5000 years. Culturally it is known as a well-being tonic to promote longevity and is considered as somewhat of a panacea [47]. In an herbal compendium published in 1955, *P. ginseng* was described as a promoter of wisdom and proposed to enlighten the mind [48,49]. Its use has become very popular worldwide and it is currently among the top ten selling herbal supplements in the US over the past decade. Ginseng is isolated from the roots of the *P. ginseng* plant and there are three commonly studied formulations: fresh ginseng, white ginseng and red ginseng. Red ginseng contains additional biological activity due to the process of generation, which involves steaming and drying of the root [50]. The most prominent phytochemicals isolated from *P. ginseng* and other *Panax* species are the saponin glycosides (ginsenosides) of which there are seven major ones, namely Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd (Figure 2) [51–56]. There are also minor ginsenosides that are characteristically found only in red ginseng such as 20(*R*)-ginsenoside Rg2 and 20(*S*)-ginsenoside Rg3. [57,58].

In addition to the ginsenosides, 15% of the *P. ginseng* root is composed of polysaccharides and 75% of those are neutral polysaccharides while only 25% are acidic [59]. The acidic polysaccharides are thought to be more biologically active; however initial studies indicated that the neutral polysaccharides contain antitumor activity [60]. Assessment of the antitumor immune function of a neutral polysaccharide fraction (WGPN) in a sarcoma S-180 tumor model determined that WGPN induced lymphocyte proliferation, increased NK cell cytotoxic activity, enhanced phagocytosis and nitric oxide production by macrophages thus increasing their cytotoxic activity, and increased TNFα secretion in the serum of tumor bearing mice [61].

Figure 2. Major ginsenosides of *Panax ginseng*. Chemical structures were taken from the PubChem Substance and Compound database (pubchem.ncbi.nlm.nih.gov). The unique chemical structure identifiers are: 441923 (Rg1-Depositor: Comparative Toxicogenomics Database) [51], 5458671 (Re/Rd mix-Depositor: MTDP) [52], 441922 (Rf-Depositor ChEMBL) [53],9898279 (Rb1-Depositor: AK Scientific, Inc., Union City, CA, USA) [54], 5458674 (Rb2-Depositor ApexBio Technology, Bristol, PA, USA) [55], 100018 (Rc-Depositor: ChemIDplus) [56].

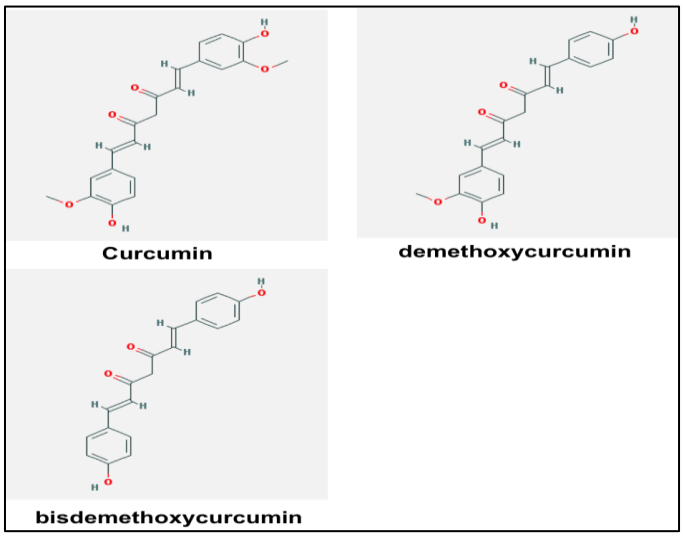


Acidic Polysaccharides of ginseng (ginsan) isolated from the ethanol insoluble fraction of the *P. ginseng* root have also demonstrated anti-cancer immune modulatory function [62–65]. Ginsan treated splenocytes isolated from unprimed normal mice became activated killer (AK) cells and could induce cytotoxic activity on a wide range of tumor cells including the NK resistant murine mastocytoma cell line P815 and NK sensitive murine lymphoma cell line YAC-1 [62,63]. Addition of recombinant IL-2 with ginsan treatment increases the generation of AK cells demonstrating that ginsan can combine with current immunotherapeutic strategies to produce enhanced antitumor effect [63]. The function of these AK cells is mediated by ginsan-produced cytokines IFNγ, IL-2, IL-1, TNFα, IL-12, GMCSF and IL-4 and the immune phenotype of these cells was determined to be $Thy1^+$ (thymocyte and peripheral T-cell marker), $AsGM^+$ (NK cell and basophil marker), $CD4^+$ and $CD8^+$. Additionally, macrophages were required as accessory cells for the generation of AK cells by ginsan [62].

Other studies indicated that ginsan treatment induced tumoricidal activity of peritoneal macrophages against murine B16 melanoma and fibroblast L929 cells suggesting ginsan's capacity to convert macrophages to an M1 tumor inhibitory phenotype [62,64]. Production of nitric oxide and reactive oxygen species by macrophages is mediated by inflammatory cytokines and ginsan treated peritoneal macrophages significantly increased their production of IFNγ, TNFα, IL-1β and IL-6 [64]. Red ginseng acidic polysaccharide (RGAP) increased cytokine production by macrophages but did not induce their tumoricidal activity on its own [65]. When used in combination with recombinant IFNγ however, RGAP had an enhanced synergistic effect on the cytokine producing, phagocytic and

cytotoxic ability of macrophages against murine B16 melanoma cells. Activation of the NFκB pathway was responsible for the anti tumor effect of macrophages treated with RGAP and recombinant IFNγ. The red ginseng ginsenoside Rg3 also demonstrates stimulatory effects on macrophages as oral administration for seven days increased the phagocytic index of peripheral blood macrophages resulting in enhanced antitumor effect in a murine non-small cell lung carcinoma model [66]. The effect of ginseng activity on macrophages extends to the immunosuppressive phenotype as Korean Red Ginseng (KRG), although having no effect on the accumulation of MDSCS, blocks their suppressive function resulting in immune activating events such as T-cell proliferation and the secretion of IFNγ and IL-2 [67]. The described studies indicate that the bioactive constituents of *P. ginseng* exhibit favorable anti-cancer immunotherapeutic effects, which are primarily mediated through generation of tumoricidal macrophages and AK cells.

Figure 3. Curcuminoids of *Curcuma longa*. Chemical structures were taken from the PubChem Substance and Compound database (pubchem.ncbi.nlm.nih.gov). The unique chemical structure identifiers are: 969516 (Curcumin-Depositor: ApexBio Technology Inc., Houston, TX, USA) [68], 5469424 (demethoxycurcumin-Depositor: Chembase.cn) [69], 5315472 (bisdemethoxycurcumin-Depositor: Chembase.cn) [70].



4. Curcumin

Curcumin, diferuloylmethane, is the active polyphenol from the *Curcuma longa* plant, commonly called turmeric, which belongs to the *Zingiberacae* (ginger) family of botanicals [71]. Turmeric is predominantly found in Southeast Asia and has been used in Ayurvedic medicine for many centuries. It has been shown to be effective against mild illnesses such as the common cold to more complex ailments as inflammatory bowel disease, pancreatitis, arthritis and cancer [72–76]. The major constituents of curcumin are called curcuminoids of which there are three phytochemicals with curcumin being the most abundant (Figure 3) [68–70]. While the other two curcuminoids demethoxycurcumin and bisdemethoxycurcumin exhibit anti-proliferative activity on various cancer cells [77–81], there are currently no studies relating to antitumor immune mechanisms of action of

these compounds which is the scope of this review. We therefore focus on the active curcuminoid curcumin, which has been used extensively in preclinical models of cancer.

Curcumin has been shown to exert a targeted immune response against several murine cancers. When used at a concentration of 25 μM, curcumin is non-toxic to immune cell types and normal non-transformed cells such as fibroblasts but exerts effective cytotoxic activity against a range of human and murine cancer cell lines such as YAC-1 murine lymphoma, human HL-60 leukemia and MDAMB breast carcinomas [82]. The *in vivo* effects of curcumin vary depending on the dose administered and doses in the range of 25 to 50 mg/kg are considered low doses and have been shown to induce significant immunostimulatory effects *in vivo* [82,83]. Wistar rats treated with 40 mg/kg of curcumin daily for 30 days generated an antigen specific T cell proliferative response to AK-5 (rat histiocytoma) tumor antigens. In a 3LL lung tumor model in mice, curcumin administered at 50 mg/kg generated a strong CTL response that involved secretion of IFNγ from activated T cells as well as suppression of immune suppressive cytokines such as IL-10 and IL-4 [83]. Interestingly, curcumin does not induce macrophage activation, as there was no effect on the secretion of ROS and nitric oxide from splenic or peritoneal macrophages. However, a delayed NK cell cytotoxic response and a temporary increase in IL-12 secretion in the serum of treated mice was observed [82]. These studies highlight the ability of curcumin to enhance the spontaneous antitumor immune response.

Several other studies explore how curcumin antagonizes the suppressive mechanisms employed by the tumor. Tumor bearing mice and cancer patients often experience a loss of both effector and memory T-cell populations, downregulation of Th1 and upregulation of Th2 immune responses and decreased proliferation of effector T-cells [84–87]. In an Ehrlich Ascites tumor model, curcumin prevented the loss of T-cells, expanded central memory and effector memory CD4 and CD8 T-cell populations in peripheral circulating blood, lymph node and at the tumor site. There was also a reversal of Th2 immune bias and increased inhibition of T-cell proliferation in tumor bearing hosts and curcumin was able to normalize the cytokine profile in the TME and dampen Treg cell activity [88].

A number of studies have elucidated the mechanism by which curcumin can restore tumor induced immune dysfunction and deregulated spontaneous antitumor immune response by the host [89–91]. Curcumin was shown to prevent T-cell depletion in both the primary and effector immune compartments of the host by inhibiting tumor secretion of the suppressive molecule prostaglandin E-2 (PGE-2) [89]. PGE-2 blocks expression of the common cytokine receptor gamma chain (γc) in T-cells which leads to deactivation of the Jak/Stat pathway and decreased expression of pro survival protein Bcl-2 in T-cells. Inhibition of PGE-2 by curcumin restores γc and Bcl-2 expression in T-cells thus promoting T-cell survival and differentiation. Tumors also secrete exosomes, which are multi-vesicular immune suppressive bodies containing a distinct set of proteins that can fuse with effector cells of the circulating immune system. Exosomes from the murine mammary adenocarcinoma cell line TS/A was shown to inhibit IL-2 mediated NK cell cytotoxicity [92]. Curcumin was shown to partially reverse the tumor exosome-mediated inhibition of NK cell function via the ubiquitin-proteasome pathway [90].

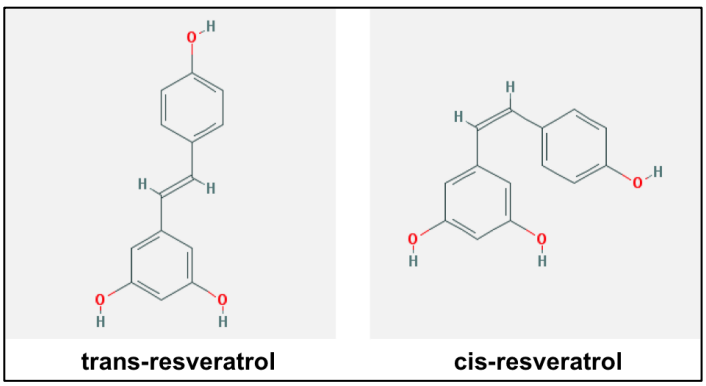
Another tumor-induced mechanism of inhibiting antitumor immune function is through the induction of oxidative stress by tumors. Tumor induced oxidative stress results in inhibition of NFκB activity in thymic T-cells, which makes the T-cells susceptible to tumor secreted TNFα induced apoptosis. This results in thymic atrophy and decreased effector T-cell populations [91,93]. Curcumin treatment prevents tumor-induced reduction of NFκB activity in thymic T cells through inhibition of

oxidative stress and decreased surface expression of TNFα receptor TNFR1 on thymic T-cells of tumor bearing mice [91]. Finally, curcumin treatment alone or in combination with adoptive T-cell therapy can inhibit the tumor suppressor indoleamine 2,3-dioxygenase (IDO) as well as the immunosuppressive cytokine TGFβ [94]. IDO exerts its immune suppressive effect by catalyzing the essential amino acid tryptophan, which is required for T-cell survival and proliferation [95]; therefore curcumin inhibition of IDO promotes T-cell cytotoxic activity. Curcumin also inhibits the two predominant suppressive immune cell types in cancer, that of MDSCs and Tregs [96,97]. Curcumin inhibits MDSC expansion and function and facilitates polarization of the MDSC population to the tumor inhibitory M1 macrophage phenotype [96]. Curcumin inhibited Treg function by blocking cell-cell contact via decreased expression of the immune suppressive receptor on T-cells Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) [97]. The work to date shows that curcumin functions as an effective antitumor drug primarily by blocking the immune suppressive mechanisms employed by tumors. This is a unique and important function of any cancer therapy and suggests that the utility of curcumin in cancer treatment needs further exploration.

5. Resveratrol

Resveratrol, 3,5,4'-trihydroxy-*trans*-stilbene, is a polyphenol that is commonly found in nature. It belongs to the stilbene class of phytochemicals and exists in both a *cis* and *trans* form (Figure 4) [98,99]. It has been identified in more than 70 plant species including common ones for human consumption such as grapes, peanuts, mulberries, cranberries and other fruits [100–103]. *Resveratrol* was found to be most abundant in the skin of grapes, which contributes to its high concentration in red wine and grape juice. *Resveratrol* is a known anti-oxidant, immunomodulator, and inducer of longevity [104–106] and has been shown to inhibit several cancer related proliferative pathways making it a promising anti-cancer therapeutic candidate [106].

Figure 4. Bioactive conformations of Resveratrol. Chemical structures were taken from the PubChem Substance and Compound database (pubchem.ncbi.nlm.nih.gov). The unique chemical structure identifiers are: 445154 (*trans*-resveratrol-Depositor: Asta Tech, Inc., Bristol, PA, USA) [98], 1548910 (*cis*-resveratrol-Depositor: Chembase.cn) [99].



Antitumor immune mechanisms of *resveratrol* are varied and in a similar fashion to curcumin they involve both immune enhancing and blockade of inhibitory mechanisms, though to a lesser extent. In a murine lymphocytic leukemia (L1210) model, resveratrol exerted a dose dependent regulatory effect

on both innate and specific immune function to L1210 bearing mice [107]. Resveratrol enhanced lymphocyte proliferation, NK cell cytotoxic activity and a specific humoral antibody response to sheep red blood cell (SRBC) as well as suppressed production and secretion of IL-6, signifying a resveratrol mediated shift to immune stimulatory antitumor responses [107]. The suppressive effect of resveratrol on anti-inflammatory cytokines is also observed in peripheral blood mononuclear cells (PBMC) [108]. PMBC treated with resveratrol exerted significant decrease in IL10, IL-6 and IL1-rα but demonstrated slight increase in secretion of pro-inflammatory cytokine TNFα. Resveratrol also suppressed the secretion of anti-inflammatory cytokines from PBMC co-cultured with colon cancer cells in a dose dependent manner, suggesting that resveratrol may induce its antitumor activity on colon cancer cells by modulating the cytokine profile of the cancer cell [108].

Inhibition of immune suppressive Treg numbers and function was observed in both *ex vivo* and *in vivo* systems [109,110] by resveratrol and involved downregulation of TGFβ secretion from the spleen of tumor bearing mice and simultaneous upregulation of IFNγ expression in CD8 T-cells leading to immune stimulation [109]. Treg expansion in the presence of high dose IL-2 (HDIL-2) treatment was blocked by resveratrol which led to abrogation of the toxic effects of HDIL-2 on endothelial cells [110]. In addition resveratrol was shown to inhibit a unique mechanism of immune suppression facilitated by the generally immune activating cytokine IFNγ. Although IFNγ is traditionally considered a pro-inflammatory cytokine with immune stimulatory activity, it is also known to be an inducer of the T cell inhibitory molecule IDO in many cell types including APCs [111]. A recent study investigated the ability of resveratrol to inhibit IFNγ induced IDO in bone marrow derived dendritic cells (BMDCs) [112]. Resveratrol mediated inhibition of EG7 thymoma tumor growth was IDO dependent and the mechanism of IDO suppression was linked to inhibition of the Jak/Stat pathway and protein kinase C δ (PKCδ) which are both required for IFNγ mediated IDO expression [113].

The most fascinating antitumor immune mechanism employed by resveratrol was very recently described and implicated the role of resveratrol in blocking breast cancer metastasis by inhibiting tumor induced regulatory B-cells (tBregs) [114]. In a previous publication, the same authors determined that tBregs promoted breast cancer lung metastasis by facilitating TGF-β mediated conversion of resting Tregs to metastasis supporting Tregs [115]. Low and non-cytotoxic concentrations of resveratrol (20 or 50 μg/mouse) significantly reduced tBregs (defined as $CD25^{+}$ $CD81^{high}$ cells within the $CD19^{+}$ population) and Tregs populations in mice. Of noteworthy mention is the finding that resveratrol had no effect on MDSCs in the tumor models and doses tested in the described study. The antitumor immune mechanisms utilized by resveratrol are multifaceted but primarily focus on inhibition of immune suppressive mechanisms resulting in enhanced antitumor effector function. Resveratrol is therefore a potent immunomodulator that can prime the TME for effective immunotherapeutic approaches.

6. Safety Data

6.1. Withania somnifera

Several studies indicate that WS does not induce severe toxicity in mice or in humans. WS was shown to be safe up to 2500 mg/kg in Balb/c mice when administered orally for seven days [116]. In

another study using the same mouse strain, the LD50 of oral administration was greater than 2 g/kg and greater than 1 g/kg for intraperitoneal (i.p.) injection. No mortality was observed during or after the treatment up to 28 days and all organs (liver, kidney, spleen, lungs, heart, stomach and intestines) were found to be normal without any atypical variation compared to control mice [41]. There was no observable change in blood pressure, heart rate, respiration rate and body temperature in rats up to 25 mg/kg after intravenous (i.v.) injection daily for 15 days [40]. Toxicity studies of steroidal lactone components of WS determined that Withanolide A is non-toxic in mice at bioactive concentrations and has an LD50 of 80 mg/kg in Balb/c mice after oral administration for seven days. Withaferin A and Withanolide D however are considered the toxic components of WS and Withaferin A has an acute LD50 of 80 mg/kg in Swiss mice [117]. The therapeutic dose (4 mg/kg) of WA however has been shown to be non-toxic in Balb/c mice [118]. A recent study to assess the safety, toxicity and tolerability of WS in 18 healthy volunteers after dose escalation of WS from 750 to 1250 mg/kg every 10 days showed no overall adverse side effects. WS was well tolerated in most participants and determined to be safe on hematological and biochemical function tests. One participant expressed increased appetite, libido, and hallucinogenic effects with vertigo after three days of WS treatment and was removed from the study. All side effects however disappeared after two days [119].

6.2. Panax ginseng

Toxicity studies involving individual phytochemicals of *P. ginseng* as well as unfractionated *P. ginseng* in mice, rats, dogs and humans have determined that ginseng exhibits limited to no toxicity in all species tested [120–122]. A randomized double blind, placebo-controlled, toxicological study determined that *P. ginseng* treatment caused no serious adverse side effects in healthy participants given 1 g or 2 g *P. ginseng* per day for 4 weeks [122]. All adverse side effects were considered mild such as insomnia, hot flash and constipation and there were no significant differences in the hematological and biochemical test results in 170 healthy volunteers.

6.3. Curcumin

Several pre-clinical trials have demonstrated that curcumin is effective against a large number of cancers including colorectal, pancreatic, gastric, prostate, hepatic, breast, oral cancers and leukemia [123]. Clinical trials involving curcumin treatment as either a single therapeutic or in combination with other agents such as *Withania somnifera* are on-going. Curcumin is also in several Phase III clinical trials which have been completed and are awaiting publication of results [124]. Phase I clinical trials determined that curcumin does not exhibit any serious toxicity in patients with adverse effects being on the level of mild nausea and diarrhea and is tolerable at doses of 3600–8000 mg daily for four months [123]. One of the main concerns of clinical advancement of curcumin is its low bioavailability as a result of its poor aqueous solubility and stability in gastrointestinal fluids [125]. Despite this concern however there has been clinical success with curcumin oral administration indicating that biologically active concentrations of curcumin and its metabolites are being retained in relevant tissues [123].

6.4. Resveratrol

Pharmacokinetic and metabolism studies have determined that resveratrol has relatively low bioavailability due to its low water solubility. Despite this, resveratrol is efficiently absorbed and exhibits high bioactivity in both human and murine studies, a phenomenon that has been described as the "resveratrol paradox" [126]. Pre-clinical and clinical studies have determined that resveratrol is safe and exhibits limited to no toxicity in mice, rats, dogs and humans [127,128]. An assessment of renal toxicity in rats given 300–3000 mg/kg resveratrol daily for 4 weeks determined that at the highest dose of 3000 mg/kg clinically significant renal lesions were observed as well as decreases in body weight and red blood cell counts [129]. The no observed adverse effect level of resveratrol is 200–300 mg/kg/day in rats and 600 mg/kg/day in dogs [127,129]. As a result of pre-clinical studies indicating the antitumor effects of resveratrol, several clinical trials against multiple myeloma, follicular lymphoma, colon and gastrointestinal cancers either alone or in combination with chemotherapeutics such as Bortezomib has been completed or are on-going [130]. Phase I trials to determine efficacy and safety show that resveratrol exhibits no serious adverse side effects at low total doses of 0.073 mg for 14 days, 100 mg for three months or 8 mg and 16 mg for six months daily. When a higher dose of 5g was administered daily for one month, resveratrol showed no serious hematological or biochemically tested adverse reactions; however mild to moderate gastrointestinal symptoms such as diarrhea, abdominal pain, nausea, cramps, fatigue and pruritus were observed [131].

7. Perspective and Conclusions

Natural compounds and their active ingredients have been the source of intense investigation as anti-cancer agents due to their multimodal action and low toxicity profiles. Dietary compounds are especially attractive as they have been in use for centuries as part of traditional diet and herbal medicines. The anticancer properties of *Withania somnifera*, *Panax ginseng*, curcumin and resveratrol have revealed multiple pathways of inhibition such as signal transduction, angiogenesis and apoptosis that affect tumor incidence and progression (Table 1). Furthermore, research on immune-stimulating effects of dietary compounds has provided a compelling link to nutrition and immunity. However the role of these agents in generating antitumor immunity has only just begun. Therefore in this review we focused on a selected set of these compounds and their constituents that have reported effects on various immune cells (Table 1) and safety profile in clinical trials.

Analyses of immunological parameters have revealed the immunostimulatory capacity of these dietary compounds primarily on NK cells, dendritic cells, macrophages and T cells. Further work is anticipated to see whether these agents can modulate other cells of the immune system such as B cells, basophils, and neutrophils. This would be beneficial as cross talk between immune cells and their soluble mediators are the key process to drive antitumor immunity. Collectively these four compounds enhance both innate and adaptive immunity and block tumor derived suppressive pathways. The reports of low systemic toxicity profiles indicate they are safe with minimal side effects. Literature survey reveals that curcumin and resveratrol have advanced to Phase II/III clinical trials for treatment of various cancers (Curcumin: NCT00094445, NCT00192842, NCT00295035, NCT00365209, NCT00118989, NCT00745134. Resveratrol: NCT00920556, NCT00455416) and therefore data

regarding their efficacy is eagerly awaited [124,130]. In addition they can be combined as adjuvant therapy or may be used synergistically with FDA approved anticancer agents [132]. However substantial hurdles exist in translation of these compounds as approved use in cancer immunotherapy.

Table 1. Summary of antitumor immune mechanisms of selected dietary compounds.

Compound	Antitumor mechanisms
Withania somnifera	▪ Withanolide A mediated Th1 polarization by secretion of IFNγ and IL-2 [41] ▪ $CD4^+$ and $CD8^+$ T cell proliferation in serum (Con A stimulation of splenocytes) [41,42] ▪ LPS induced B-cell proliferation from splenocytes [42] ▪ Generation of *de novo* T cells (thymocyte proliferation) [42] ▪ NK cell activation (ADCC mediated cell death) [42] ▪ DC activation and maturation [41] ▪ WA mediated tumoricidal macrophage activity [43] ▪ WA induced immunogenic cell death-authors unpublished results [44] ▪ Inhibition of MDSC activity in TME [46]
Panax ginseng	▪ T-cell proliferation, NK cytotoxicity and macrophage activation by neutral polysaccharides [61] ▪ Ginsan mediated generation of Activated Killer cells [62,63] ▪ Ginsan and Rg3 mediated generation of tumoricidal macrophages [62,64,66] ▪ Red ginseng acidic polysaccharide in combination with recombinant IFNγ induces tumoricidal macrophages [65] ▪ T-cell activation by inhibition of MDSC activity [67]
Curcumin	▪ Cytotoxic T lymphocyte (CTL) activity via increased IFNγ secretion [82] ▪ Restores T-effector and central memory of CD4 and CD8 T-cell population systemically and in TME [88] ▪ Inhibit Treg function [88] ▪ Polarizes cytokine profile of TME to Th1 responses [88] ▪ Inhibits of MDSC expansion and function. Converts MDSC to M1 tumor inhibitory phenotype [96] ▪ Inhibits Treg accumulation and function [97] ▪ Inhibition of prostaglandin E2 (PGE-2) activity [89] ▪ Reversal of tumor exosome mediated inhibition of NK cell function [90] ▪ Prevents thymic atrophy induced by tumor mediated oxidative stress and restores TNFα mediated CTL function [91] ▪ Inhibition of indoleamine 2,3-dioxygenase (IDO) and TGF-β [94]
Resveratrol	▪ Induces T cell proliferation [107] ▪ Activates NK cytotoxic function [107] ▪ Induces antigen specific B-cell response [107] ▪ Inhibits IL-6 secretion [107] ▪ Suppresses anti-inflammatory cytokine secretion by cancer cells interacting with PBMCs [108] ▪ Inhibits accumulation and function of Tregs [109] ▪ Blocks tumor promoting IFNγ mediated IDO expression in bone marrow derived dendritic cells [112] ▪ Inhibits expression and function of tumor induced Bregs and metastasis promoting Tregs [114]

Dietary compounds have a complex of chemical structures that were selected evolutionarily in comparison to synthetic drugs. Although this is beyond the scope of this review, pleiotropic effects such as immune-enhancing and immune-suppressive functions of these compounds should be noted. For example both curcumin and resveratrol induce tolerogenic DC and terminate activation [133]. This intuitively leads us to question the role of these various biologically moieties in the immune system

and will require further research to sort out the opposing effects. The next challenge is to address the short half-life and low bioavailability due to the poor water solubility of the active ingredients. Ongoing research such as encapsulation in biodegradable PLGA nanoparticles or sustained release in water insoluble cellulose acetate matrices increase the bioavailability and half-life of bioactive forms of dietary compounds. Thus the future research should not only define various antitumor mechanisms of these dietary compounds but also address technological advances for proper delivery to the site of tumor to modulate TME that should include a reliable pharmacological read out of the physiologic dose required to generate antitumor immunity.

Acknowledgments

The unpublished work on Withaferin A is funded by F31 pre-doctoral fellowship, NCCAM (A.S.) and American Cancer Society Grant (S.A.). We specifically acknowledge the curators and depositors of the PubChem Substance and Compound database (pubchem.ncbi.nlm.nih.gov) for the structures of the compounds.

Conflicts of Interest

The authors declare no conflict of interest.

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