Phytosterols in cancer: From molecular mechanisms to preventive and therapeutic potentials

Running title: Role of phytosterols in cancer prevention and therapy

Authors: Francisco Blanco-Vaca* †, Lídia Cedó*, Josep Julve†

Author affiliation: Institut d'Investigacions Biomèdiques Sant Pau (IIB-Sant Pau), Barcelona, Spain (FB-V, LC, and JJ). CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Madrid, Spain (FB-V, LC, and JJ). Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain (FB-V, and JJ).

* These authors contributed equally to this work.

† These authors are co-corresponding authors of this work.

Address correspondence to: Dr. Francisco Blanco-Vaca or Josep Julve, IIB-Sant Pau, C/Sant Antoni M. Claret 167, 08025 Barcelona, Spain

Abstract

Cancer is the second leading cause of death worldwide. Compelling evidence supports the hypothesis that the manipulation of dietary components, including plant compounds termed phytochemicals, demonstrates certain important health benefits in humans, including those in cancer. In fact, beyond their well-known cardiovascular applications, phytosterols may also possess anticancer properties, as has been demonstrated by several studies. Although the mechanism of action by which phytosterols (and derivatives) may prevent cancer development is still under investigation, data from multiple experimental studies support the hypothesis that they may modulate proliferation and apoptosis of tumor cells. Phytosterols are generally considered safe for human consumption and may also be added to a broad spectrum of food matrices; further, they could be used in primary and secondary prevention. However, few interventional studies have evaluated the relationship between the efficacy of different types and forms of phytosterols in cancer prevention. In this context, the purpose of this review was to revisit and update the current knowledge on the molecular mechanisms involved in the anticancer action of phytosterols and their potential in cancer prevention or treatment.

Keywords**:** edible vegetables – sitosterol – antitumor therapies – angiogenesis – proliferation – metastasis – apoptosis – phytotherapy

1. Introduction

Cancer is the second leading cause of death and morbidity worldwide, accounting for 8.7 million deaths in 2015 (*1*). As per the projections for 2030, these figures will double by the year 2030 (*2*).

The pathophysiology of cancer is complex; however, it may be defined as uncontrolled cell growth that may spread to other body parts from the original site, resulting in tissue disorganization and destruction of these other body parts (*3*). This change is attributable to the dysregulated expression of genes involved in cell cycle control (*4*). Conventional therapies frequently fail, partly because they generally target single processes, often causing adverse health effects.

Diet has been identified as an important and modifiable risk factor for cancer (*5-7*). Therefore, modification in dietary habits that involves the inclusion of functional food components with chemopreventive properties has been identified as a potential strategy for halting or reversing the early phases before the manifestation of a malignancy. Supporting this theory, diets high in fruits and vegetables, legumes, and whole grains are chemopreventive (*8, 9*). Moreover, there is considerable epidemiologic data suggesting that vegetarians tend to have lower overall rates of cancer than the general population (*4*), being one of the most convincing data coming from long-term association studies of the Seventh-Day Adventist community (*10, 11*). Further, adherence to the traditional Mediterranean diet has also been evidenced to be protective against the risk of cancer, mainly owing to its higher content of abundant and variable plant foods, high consumption of cereals, and predominant use of olive oil as the main fat (*12-14*). Therefore, it is not surprising that the intake of plant-origin foods appears in the current guidelines as one of the main evidence-based recommendations for reducing the risk of cancer (*2, 15-18*). Overall, these studies further reveal the presence of dietary functional components with cancer-preventive potential in these diets (*19*).

The functional components of food can be defined as non-conventional biomolecules present in foodstuffs that are closely associated to health benefits (*20*). Research has proved that functional dietary components can be effectively used in the treatment and especially, in the prevention of diseases. Studies have shown that supplementation with specific plant metabolites (e.g., antioxidants, dietary fiber, and different species of phytochemicals) may reduce the individual risk and alleviate the risk of specific types of cancers (*2*). In particular, in terms of anticancer therapies, dietary phytochemicals have received increasing attention because of their high potency and low toxicity in modulating crucial intracellular signaling pathways than the common chemotherapy agents (*21*). Phytosterols are one such class of

bioactive dietary phytochemicals (*4*). This class of molecules has been shown to be effective in improving the blood lipid profile, thereby protecting against cardiovascular disease. In addition to their cardioprotective effects, phytosterols have also shown to have a protective effect against different forms of cancer (*22*). These compounds are naturally present in Western diets in amounts similar to those of dietary cholesterol (*23*); the amount of these compounds in vegetarian diets is about 50% higher (*24*). In this context, we herein will critically review the potential role for dietary phytosterols in cancer prevention.

2. Dietary phytosterols

2.1. Molecular characteristics

Phytosterols are the plant equivalents of animal cholesterol. These compounds are structurally similar to cholesterol except for the extra double bonds and methyl and/or ethyl groups in their side-chain (*25*). More than 200 phytosterol species exist naturally in plants, and many phytosterols are found in edible foodstuffs (*25*). In foodstuffs, the most abundant dietary phytosterols are β-sitosterol, campesterol, and stigmasterol, with the prevalence of β-sitosterol being particularly noteworthy (*4*). Phytostanols, saturated phytosterols, are less abundant than phytosterols (*25*). Stanol species can be produced by 5-alpha hydrogenation of the corresponding phytosterols (e.g., sitostanol and campestanol) (*26*).

2.2. Sources

In human beings, the diet is the only source of phytosterols. In foods, total phytosterol content is defined by the sum of free sterols as well as the conjugated (*i.e.*, esterified and glycosylated) forms (*25, 27*). The common dietary sources of phytosterols are vegetables, grains, seeds, and vegetable oils. The content of phytosterols is high in lipid-rich plant foods, such as nuts, legumes, and seeds (*4*). Similarly, many fruits and grains also contain significant levels of phytosterols although usually at lower concentrations. Additionally, industrial procedures used in food production further affect the phytosterol content of plant foodstuffs. For instance, refining processes, as those involved in the production of refined olive oil (*27*), can result in significant reduction in their relative phytosterol content. Environmental conditions may also influence the phytosterol content and the composition of conjugated forms (*4, 27*). Phytosterols are found in many foods and are currently a part of the normal human diets. All plant foods, including algae (*28*), contain phytosterols (*29, 30*). In the context of the food industry, phytosterols are currently added to a broad spectrum of foods other than fat spread; they are also added to low-fat milk, bakery products, orange juices, cereal and chocolate bars, as well as low-fat beverages (*30, 31*).

2.3. Bioavailability

An important issue related to the potential health benefits of phytosterols is their bioavailability. The main bulk of phytosterols is solubilized in micelles that enable their absorption and transport through the same transporter used for cholesterol transport (*23*), the Niemann Pick C1-Like 1 transporter. Similar to free cholesterol, non-esterified phytosterols may also accumulate within enterocytes or be rapidly re-secreted into the intestinal lumen via ABCG5/G8 transporters (*23*). Moreover, phytosterols, which are not fully metabolized by the human body, are rapidly excreted, eventually resulting in a minimal net absorption of the dietary phytosterols. Despite this, measurable circulating levels of phytosterols can be detected, especially in individuals who consume phytosterol-enriched diets (*27, 30*).

Several factors may potentially influence phytosterol absorption (*30, 32*). In human beings, the absorption efficiency for phytosterols is considerably lower (from less than 5 to approximately 10% depending on the study) compared to that of cholesterol (33-60%) (*27, 33-39*). Dietary phytostanols are absorbed more poorly absorbed than phytosterols (*34, 40*). Phytosterol absorption may be also influenced by the quantitative differences in the common food sources (*30, 32, 41*). The bioavailability of phytosterols is enhanced in oils (unrefined, vegetable, and olive oils) (*42*). The phytosterol content in foodstuffs may also be reduced by the production processes, including refining of crude edible oils to remove the known contaminants extracted from raw foodstuff or hydrogenation to obtain stanol-enriched food matrices. Further, boiling and storage variably affects the total phytosterol content of vegetables (*41*). As in the case of cholesterol, thermo-oxidative treatments during food processing or oxidation that occur during storage in bulk oil, oil-in-water emulsions, and infant foods may lead to the formation of phytosterol oxidation products (*i.e.*, keto-, hydroxyl-, and epoxy-derivatives) (*30, 41, 43*). Furthermore, an increased dietary exposure to these oxidized constituents via the consumption of foods enriched with phytosterols/phytostanols may potentially influence the absorption and hamper the biological properties attributed to phytosterols (*44*).

Liposomes are carriers that improve the molecular stability and permeability and control their release into the target tissues. Liposomalization has been reported as another strategy for oral administration (*45*). This approach improves the bioavailability of several substances that are insoluble or barely soluble in water. To our knowledge, only one study has reported on the effect of oral administration of liposomal β-sitosterol in preventing tumor metastasis *in vivo* (*46*), suggesting that liposomal-based vehicles could be potentially good candidates for formulating phytosterols in cancer prevention or treatment.

2.4. Safety

Studies on phytosterols have been conducted on several population groups, including subjects from Europe, Americas, and Asia (*47*). No adverse effects have been reported in any of these clinical studies or by the regular consumption of food products containing these compounds. Compared to phytosterols, phytostanols are more stable and are therefore not altered during food manufacturing or processing. These compounds are microbiologically inert because they are not affected by fermentation and are not oxidized when heated (*48*). In a similar manner, they are not metabolized in the body and are excreted intact (*49*). Unabsorbed phytosterols can further undergo bacterial transformation by the intestinal microbiota to produce metabolites, such as coprosterol and its immediate precursor, coprostanone (*50*). Although these metabolites are currently used as biomarkers for the presence of human fecal in the environment, some reports have proposed their potential as biomarkers for certain types of cancer (*i.e.,* colon cancer) (*51-53*). Consumption of phytosterols hampers the intestinal cholesterol absorption and might also interfere with the appropriate intake of other dietary fats, including fat-soluble vitamins (α-tocopherol) and carotenoids (α-carotene, β-carotene, and lycopene) (*54*). Given their role in antioxidant prevention, reduced plasma levels of these molecules might have negative health implications. Different meta-analyses of randomized controlled trials have revealed that phytosterols intake significantly reduced plasma levels of α-tocopherol, α-carotene, β-carotene, and lycopene compared to subjects administered placebo (*55, 56*). These molecules are transported by lipoproteins; therefore, a reduction in their plasma levels might be attributable to the reduced capacity of the plasma carriers (*57, 58*). Thus, their plasma levels are generally standardized for total cholesterol (*55, 56*). After correction, only the plasma carotenoid concentrations were eventually found lower after phytosterol intake (*56*). However, the observed levels of fat vitamins were maintained within the normal ranges, suggesting that it may have no health impact.

3. Cancer prevention properties of dietary phytosterols

Several studies have shown a direct relationship between dietary habits and the incidence of cancer (*5-9*). Representative epidemiologic data support the results that the prevalence of cancer is frequently lower in representative vegetarians (e.g., Adventists) than in the general population (*10, 11*). In this regard, the estimated dietary intake of phytosterols of the Adventists is reportedly higher than that of the general population (*59*). Similarly, the naturally-occurring dietary intake of phytosterols in a Spanish free-living population has been also estimated to be higher than that of people living in other non-Mediterranean European countries (*60*), suggesting that this could be a part of the Mediterranean diet. A randomized,

clinical trial involving a Mediterranean diet intervention showed a reduction in the breast cancer risk of the intervention group (*61*). Although the specific contribution of the main component(s) responsible for the favorable anti-cancer effect of plant-based diets remains undefined, it can be supposed that the cancer protection provided by these diets can be attributed, at least in part, to their increased phytosterol content (*13, 62*). Probably the most convincing experimental data supporting this hypothesis is that from the efficacy analysis of this class of molecules on cancer protection in animal models of different types of cancers (*4*).

In the last years, a growing body of experimental evidence has supported the hypothesis of the protective effects of phytosterol consumption against tumor growth and tumor cell metastasis (Figure 1). Most studies have been conducted on cancer cell lines *in vitro* (*63*). In contrast, relatively fewer *in vivo* studies on the antitumor effect of phytosterols have been conducted, most of them using different rodent models of chemically-induced cancers, xenographed with cancer cells, (*3, 4*) or genetic models of breast and prostate cancer (*64, 65*).

The anti-proliferative and anti-metastatic effects of phytosterols (*i.e.*, either β-sitosterol or other phytosterol mixtures) support the epidemiological studies that suggest a protective role of phytosterols against the development of various cancers.

4. Potential cellular mechanisms of anticancer action

The exact mechanisms involved in the phytosterol-mediated protection against cancer are still poorly defined. Different potential anticancer mechanisms have been proposed, including those involving the inhibition of carcinogen production, cancer cell proliferation, invasion, and metastasis, as well as the induction of cell cycle arrest and apoptosis (reviewed in (*3, 4, 22, 66*), thus conferring cancer chemopreventive and therapeutic potential. Apart from these, other mechanisms including the reduction of angiogenesis, invasion, and adhesion of cancer cells have been also suggested (Figure 1).

4.1. Effect on cell cycle

Cell cycle dysregulation may be mainly attributed to the alterations in certain control proteins. Phytosterols have been reported to reduce cell cycle kinetics in different cultured cancer cell lines (*4, 66*). These compounds may potentially act as weak cell cycle inhibitors of cancer cells. Compelling experimental evidence from different studies suggests that exposure to phytosterols (*i.e.*, β-sitosterol) may either slow down cancer cell growth, as revealed by cell cycle arrest at different phases, depending on the study (*i.e.,* G2/M and G1/S transitions, G0/G1, or G2 phases) or promote cell accumulation of the sub-G1

apoptotic population (*4, 67-72*). In this regard, pretreatment of a rat model of renal carcinogenesis with β-sitosterol has proven effective against renal cancer, partly owing to the inhibition of cellular proliferation; hence, a significant reduction in the gene expression of proliferative markers (*i.e.*, cyclin D and Proliferating Cell Nuclear Antigen [PCNA]) has been concomitantly observed in renal biopsies (*73*). The mechanisms involved in cell arrest triggered by phytosterols are still poorly defined.

4.2. Effect on apoptosis

The most accepted hypothesis is that phytosterol supplementation at physiological concentrations ultimately inhibits cancer cell/tumor growth through apoptosis stimulation [4]. Several researches have shown that β-sitosterol promotes apoptosis in many different types of cancers (*4, 68-71, 74-79*). While the mechanisms involved in the apoptotic action of phytosterols are not yet fully understood, some progress has been made in this field. In this context, β-sitosterol exposure to breast MCF-7 and MDA-MB-231 adenocarcinoma cells has been found to increase First apoptosis signal (Fas) levels and caspase-8 activity that are involved in the extrinsic apoptotic pathway (*77, 79*). The increase in the caspase-8 activity and apoptosis in these cells is accompanied by an enhanced deposition of β-sitosterol in their cell membranes (*79*). This finding suggests a potential mechanism by which β-sitosterol might induce apoptosis, at least in part, by changes to the membrane sterol content and the Fas apoptotic pathway (*79*). In addition, the activity of other caspases (*i.e.*, caspase-3 and -9) also increases in colon cancer cells, following incubation with β-sitosterol (*76*). The latter has been associated with the down-regulation of the anti-apoptotic protein Bcl-2 (*80*) and up-regulation of the pro-apoptotic Bax, an activator for ceramide-mediated apoptosis (*81*), as well as with a release of cytochrome C from the mitochondria that is involved in the intrinsic apoptotic pathway. In a similar manner, β-sitosterol modulated Bax/Bcl-2 proteins and caspase-3 and caspase-9 *in vitro* in leukemic cells (*70*) and stomach cancer cells (*68*) and *in vivo* in a rat model of renal carcinogenesis (*73*). Similar effects are produced by 7α-hydroxy-β-sitosterol, a rare phytosterol oxide isolated from *Chisocheton tomentosus* with the ability to dysregulate the Bax/Bcl-2 ratio (*82*) or guggulsterone that is found in the resin of *Commiphora mukul* and induces apoptosis through the intrinsic mitochondrial pathway in hepatic carcinoma (*83*) and cholangiocarcinoma cells (*84*). Both apoptotic pathways (extrinsic as well as intrinsic) are also involved in the induction of apoptosis by β-sitosterol in fibrosarcoma cells (*75*). In this case, β-sitosterol reduced the cellular levels of Bcl-2 and the inhibitor of the apoptosis protein (IAP) family and increased Bax, p53, and p21. It is interesting to note that this phytosterol also reduced the activity of known anti-apoptotic molecules (*i.e.*, PI3K and Akt) and

increased the activation of pro-apoptotic signal transduction enzymes, including the extracellular signal-regulating kinase (ERK) and p38 mitogen activated protein kinase that are activated (via phosphorylation) due to extracellular signals and are involved in the activation of caspases (*75*).

4.3. Effect on metastasis

Metastasis is one of the most threatening aspects of cancer (*85*). Despite major advances in cancer treatment, there are still no specific treatments to prevent metastasis. This is because metastasis is a multistep process that comprises certain processes, including tumor cell migration, invasion, adhesion, and growth.

Dietary phytosterols (*i.e.*, β-sitosterol) have been reported to exert a protective effect against prostate and breast cancer metastasis in immunodeficient (SCID) mice (*71, 86*). Mice (male) inoculated with cultured prostate cancer cells (PC-3 type) and fed with phytosterols exhibited (37%) about one-half the rate of metastasis than cholesterol-fed mice (73%), with fewer cases of metastases to the lymph nodes and the lungs (*86*). Furthermore, mice treated with phytosterols did not present detectable signs of metastasis in the liver compared to the 18% prevalence detected in the control mice with tumors. In keeping with this, a study based on the same mouse model as the one described above for prostate cancer but using SCID mice (female) inoculated with cultured breast cancer cells (MDA-MB-231 type) and fed phytosterols also exhibited fewer cases of metastases to the lymph nodes and the lungs (52%) compared to the control group (71%). Investigations regarding the metastatic step targeted by phytosterols have found that β-sitosterol offered protection from breast cancer metastasis by inhibiting cell invasion and adhesion to the basement membrane proteins (*87*), potentially by directly influencing the expression of integrin receptors on the tumor cells. It is noteworthy that, in the same study, campesterol (another phytosterol examined in the study) failed to protect against metastasis.

More recently, orally administered liposomal β-sitosterol also prevented lung metastatic colonization of melanoma cells in an experimental metastasis model *in vivo* (*46*). In this study, β-sitosterol induced IL-18 production in the intestinal epithelium. IL-18 modulates the immunological function and enhances natural killer action. Since β-sitosterol is absorbed in very small amounts into the plasma, the authors hypothesized that the stimulation of mucosal immunity rather than a direct action on the tumor cells provided protection against tumor metastasis.

4.4. Effect on angiogenesis

Angiogenesis is required for invasive tumor growth and metastasis and is critical in the control of cancer progression (*88*). The angiogenic response involves many molecules and signaling pathways that coordinate multiple cellular processes, such as endothelial proliferation (*i.e.*, vascular endothelial growth factor [VEGF] or its receptor(s), basic fibroblast growth factor [bFGF], etc.), and basement membrane remodeling (*i.e*., matrix metalloproteinases [MMP], such as MMP-2 and MMP-9) (*66*). Tumoral microenvironments may also be extremely hypoxic; the activation of hypoxia-dependent signaling pathways, especially hypoxia-inducible factor (HIF)-1, further contribute to tumor angiogenesis (*89*). In this context, the inhibition of cancer angiogenesis may be a valuable, new approach to cancer therapy.

The anti-angiogenic potential of phytosterols remains controversial. Although data first suggested that β-sitosterol possesses good angiogenic activity both *ex vivo*, using a chick embryo chorioallantoic membrane (CAM) assay, and *in vivo* using a model of ischemia/reperfusion of vascular damage (*90, 91*), some recent evidences suggest the mechanism by which phytosterols potently inhibit tumor neovascularization (*3*). For instance, oral pretreatment of β-sitosterol has been reported to reverse the VEGF gene expression in a rat model of renal carcinogenesis (*73*). The administration of another main phytosterol, campesterol, has been shown to prevent bFGF-induced endothelial cell proliferation and capillary-like tube formation as well as neovascularization in fertilized eggs (*92*). Guggulsterone has been recently reported to suppress capillary tube formation and the migration of endothelial vascular cells as well as matrix metalloproteinases release from the colon cancer cells (*66*). The anti-angiogenic activity of phytosterols, as one of the components of a dietary phytochemical conjugate, has been also demonstrated *in vivo* using a murine Lewis lung cancer model (*93*). Further, it is important to note that the reduction of tumor-associated angiogenesis and growth observed in conjugate-feeding mice were not exclusively attributed to the phytosterol content (*i.e.*, soy sterols) because other plant bioactive components with antitumoral potency were also present in the conjugate formula (*i.e*., lipoic acid and ferulic acid) (*94, 95*). However, these effects were similar to the reported effects of these compounds and support the usefulness of combined dietary approaches in cancer prevention.

4.5. Effect on immunomodulation

Selective modulation of the different components of the immune system has received considerable attention because it forms the treatment bases for several pathological conditions, including cancer (*96*). It is generally accepted that immune surveillance mechanisms are vital for preventing the development of solid tumors. Such mechanisms depend heavily on a well-balanced immune response favoring a cellular

outcome (T helper 1 [T_{H1}] mediated) rather than a humoral response (T_{H2} type) (*97*). The immunoregulatory role of phytosterols is deeply reviewed in another article of this special issue.

5. Potential biochemical mechanisms

5.1. Cholesterol-lowering properties

Elevated plasma cholesterol levels lead to their accumulation in the cell membranes where they concentrate into particular cell membrane structures termed as lipid rafts. Increased cholesterol content has been associated with increased survival and reduced apoptosis of cancer cells (*98*). Further, high intakes and elevated blood levels of cholesterol are believed to be associated with elevated cancer risk (*99-101*). According to this, the use of cholesterol-lowering strategies, including phytosterols, could reduce the risk of cancer. In fact, the use of statins, another cholesterol-lowering therapy, has been experimentally demonstrated to have an anti-cancer effect (*102*). Consistently, most existing epidemiologic evidence supports a survival benefit of statins in oncological patients (*103-105*). Thus, it is not completely accurate to believe that lowering the plasma cholesterol levels would result in phytosterol-induced depletion in the cholesterol in the lipid rafts of cancer cells, and thereby induce cell death by regulating signal transduction (*3*). This might further suggest a potential raft-modulating action that involves the incorporation of phytosterols into the lipid rafts and a potential use for these compounds as anti-cancer agents.

5.2. Modulation of cell signaling

5.2.1 Liver X receptor (LXR)

LXRs also act as oxysterol sensors in the body and their potential therapeutic applications have been examined in several types of cancers (*106*). Phytosterols have been shown to activate the LXR signaling *in vivo* (*4, 107*). However, the mechanisms involved in the LXR-mediated anti-cancer effect by phytosterols are still poorly defined. The activation of LXR enhances cholesterol removal from the tissues (*108*), and it has been proposed that this may directly contribute to further enhancement in cholesterol removal from the cancer cells. In addition to its potential direct impact on cellular cholesterol, its activation by ligands (*i.e.*, T0901317) has been also shown to exert anti-proliferative actions in multiple cancer-related study systems (*106, 109-112*), potentially by promoting cell cycle arrest at the G1 phase (*112*). In this context, the overexpression of LXRα further sensitizes the cancer cells to the effect of T0901317 (*113*). The activation of LXRα with other agonists has been also reported to have a protective effect against proliferation (*i.e.*, GW3965) and progression of either prostate or colorectal cancer in tumor

xenograft models *in vivo* (*110, 112*). Supporting this notion, the treatment with LXR ligands induces the degradation of the low-density lipoprotein receptor (LDLR), a regulator of tumor cell survival, and increases apoptosis in glioblastoma cells (*106*). Further, LXR-mediated elevation in apolipoprotein E gene (*Apoe*), another known LXR target, promotes metastasis suppression in melanoma models (*106*). These findings might further highlight the role for phytosterols in the inducing APOE-mediated mechanisms in cancer therapy.

Phytosterol oxidative derivatives, called oxyphytosterols, may also act as LXR activators (*107*), and they exert significant antiproliferative effects on cancer cells (*114*). Although it is still unclear whether oxyphytosterols are formed in the relevant concentrations *in vivo* (*107*), significant amounts of these compounds are present in foods (*30, 41*) and may be detected in the plasma of healthy subjects (*115*). Taken together, this evidence strongly supports a link between LXR function and cancer. However, it is still uncertain whether the consumption of natural dietary phytosterols might eventually lead to the activation of LXR *in vivo*. Deeper investigation (*i.e.*, loss-of-function experiments) is needed in order to confirm the role of LXRs for the chemopreventive actions of phytosterols in cancer.

5.2.2. Sphingomyelin cycle

One of the anti-cancer mechanisms of phytosterols that has received the maximum attention is the induction of the sphingomyelin-based signaling pathway (*116, 117*). Ceramides as well as other sphingolipid metabolites derived from sphingomyelin can modulate cell growth, survival, and death (*118, 119*). A significant induction of cellular apoptosis has been observed in MDA-MB-231 breast adenocarcinoma cells, LNCaP prostate adenocarcinoma cells, and colon cancer cells exposed to β-sitosterol (*71, 78, 116, 117*). It is important to note that the phytosterol-mediated promotion of apoptosis seems to be selective for cancer cells because exposition to β-sitosterol does not promote apoptosis in normal cells.

5.3. Role of phytosterols in oxidative stress

Oxidative stress is closely related to cancer (*120*). Cancer cells require a high amount of ATP for aberrant proliferation. This high demand of energy is uncontrolled and leads to the accumulation of reactive oxygen species (ROS); this needs to be counteracted by antioxidant mechanisms to ensure cell survival, even in cancer cells. Current data support the hypothesis that low to moderate levels of ROS may contribute to tumor formation either by activating the signaling pathways or by promoting the mutation of genomic DNA (*120*). By contrast, at high levels, ROS promote cell death and severe cellular damage.

Thus, cancer cells need to develop powerful antioxidant mechanisms to counteract the exacerbated levels of oxidative stress produced at the early stages of tumor development and during the metastatic phases of the disease.

β-sitosterol has been reported to elevate the activities of antioxidant enzymes in cultured macrophage cells with phorbol 12-myristate 13-acetate-induced oxidative stress, suggesting that phytosterols can protect cells from damage by ROS (*121*). β-sitosterol has been also reported to decrease free radical generation *in vitro* (*122*). Consistent with these findings, the administration of β-sitosterol has been recently reported to exhibit chemopreventive potential in 1,2-dimethylhydrazine (DMH)-induced experimental colon cancer in rats by reducing the oxidative stress (*123*). Although current data supports the theory that phytosterols exert direct antioxidant effects (*124, 125*), recent data further reveal that the phytosterols may also exert pro-oxidant effects by triggering ROS-dependent apoptosis and DNA damage in multiple myeloma cells (*67*). This phytosterol-mediated action was attenuated by the ROS scavenger N-acetyl L-cysteine and was associated with the activation of several cancer metabolism-related signaling pathways, such as AMP-activated protein kinase and c-Jun N-terminal kinase. The latter study contradicts the hypothesis regarding the complex role of natural phytochemicals, including phytosterols, and their dual antioxidant or pro-oxidant role in early or metastatic events of chemoprevention and cancer therapy.

Lipoprotein oxidation, critical for tumor growth (*126, 127*), is increased in PyMT mice (*128*). Data from our group indicate that phytosterols were effective in preventing lipoprotein oxidation in mice fed a high-fat diet; this may explain, at least in part, their anticancer effects observed in these mice (*64*). In this regard, it is noteworthy that lovastatin, a member of the statin family, inhibits low-density lipoprotein oxidation (*129*). This property could be, at least in part, involved in the anticancer activity attributed to this category of drugs.

6. Potential of phytosterols as anticancer agents

Although data on pharmacokinetic and bioavailability has been previously reported (*130*), few reports have described the β-sitosterol dosage forms for their potential application in cancer cell lines. The absorption of dietary phytosterols is only 5%; therefore, the phytosterol concentration used in *in vitro* studies is generally higher than that actually available to cells *in vivo*. However, these studies have proven useful for the investigation of the molecular mechanisms involved in the inhibitory effect of phytosterols on tumor growth.

The anticancer and antiapoptotic potential of phytosterols is currently under investigation. Several trials have suggested that their efficiency may be mainly revealed in combined therapies for cancer treatment (*74, 131*). These findings seem to indicate that their action may rather depend on the targeted cell type, source, or concentration of these compounds. Consistent with this finding, β-sitosterol has shown to become pro-apoptotic in human stomach cancer cells (SGC-7901) (*68*); however, it has failed to show effectiveness in human intestinal caco-2 cells (*132*). Moreover, in independent studies, only high concentrations of β-sitosterol have shown to induce apoptosis (*77, 133*).

To the best of our knowledge, no clinical trials have been conducted to test the potential efficacy of phytosterols in cancer. In general, epidemiological studies have reported that phytosterol intake is beneficial in reducing the risk of cancer in the lung (*134*), breast (*135*), esophagus (*136*), stomach (*137*), endometrium (*138*), and ovaries (*139*). A recent case-control study has shown that an increased intake of dietary phytosterols, especially β-sitosterol, campesterol, and campestanol has been associated with a 50% reduction in the risk of colorectal cancer in a Chinese population (*140*). Although, to our knowledge, there are no clinical studies linking β-sitosterol and breast cancer, its administration may enhance the efficacy of tamoxifen, a drug commonly used for the treatment of breast cancer (*131*). Although phytosterol administration is generally effective against various forms of cancer, some recent studies have reported contradictory results. For example, in a study conducted in the Netherlands, phytosterol intake was not associated with a lower risk of colon and rectal cancer (*141*). It is noteworthy that there was a positive association of campesterol and stigmasterol with the risk of prostate (*142*) and colorectal cancer (*140*). Thus, their value (alone or in combination with other anticancer treatments) in cancer prevention needs to be confirmed in future controlled human trials.

Multidrug resistance is a phenomenon wherein tumors become resistant to chemically unrelated anticancer drugs, posing one of the most important challenges in the field of cancer chemotherapy. One of the most common mechanisms leading to multidrug resistance is the increased efflux of cytotoxic drugs by energy-dependent transporters (ABCs). Recent studies have documented the ability of natural phytochemicals to increase the sensitivity of cancer cells to anticancer drugs (*143*). In particular, the efficacy of phytosterols has been proven in the treatment of multidrug resistant cancer cells at physiological concentrations (*72*). It is noteworthy that in the same study, β-sitosterol exhibited greater cytotoxicity in the cells with basal expression of ATP-binding cassette, sub-family B, member 1 (ABCB1) that is responsible for multidrug resistance phenomena, than in multidrug resistant cells. The

exact mechanism of phytosterol action in cancer cells is still unclear; however, it has been postulated that phytosterols can exert their action partly by inhibiting the action of ABCB1 (*72*). This study also suggested that phytosterols can target cancer cells, exhibiting high multidrug resistance potential.

7. Concluding remarks

As per most experimental studies, phytosterols are potential agents for prevention and, perhaps, for the treatment of cancer. As a natural product, at concentrations present in plant sources or formulas, phytosterols have little or no reported toxicity, making them a promising treatment option for several types of cancer. The major dietary phytosterols are β-sitosterol, campesterol, and stigmasterol, and their contents are higher in edible oils, seeds, and nuts. Several experimental and few human clinical studies have also revealed their potential therapeutic value in cancer prevention. In particular, the characterization of phytosterols as LXRs ligands as well as their effects and mechanisms of action in preclinical cancer models may readdress future translational LXR research in cancer therapeutics. Moreover, new leads in the development of new phytosterol derivatives (or analogs) or suitable drug delivery systems for targeted therapy might provide greater efficacy and bioavailability, thus improving their clinical usefulness for human trials. Future studies are therefore warranted to investigate the detailed mechanisms of action of phytosterols *in vivo* as well as to assess the long-term effects of these compounds on the prevention of tumor initiation, growth, and angiogenesis.

Abbreviations used: ABCG5/G8, ATP-binding cassette transporters class G type 5 and 8; bFGF, basic fibroblast growth factor; CAM assay, (In Ovo) Chick Chorioallantoic Membrane assay; LXR, liver X receptor; PCNA, proliferating cell nuclear antigen; MMP, metalloproteinases; PyMT, polyoma middle T antigen; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; WHO, World Health Organization.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgements

F.B.-V., L.C., and J.J. have contributed in the editing and revision of the manuscript. This work was partly funded by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (ISCIII) FIS grants

PI17/00232 (to JJ), PI14/01648 (to FB-V), and FEDER “Una manera de hacer Europa” and by LaMarató 2016; grant number 201602.30.31 (to JJ). J.J. is recipient of a Miguel Servet Type 1 contract (CP13/00070; ISCIII). CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM) is a project of the Instituto de Salud Carlos III. The English grammar and language were corrected by Scribendi Inc. (www.scribendi.com). Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau is accredited by the Generalitat de Catalunya as Centre de Recerca de Catalunya (CERCA).

Figure legend

Figure 1. Anticancer mechanisms and molecular targets of phytosterols in cancer prevention and therapy. Phytosterols affect the signaling pathways involved in carcinogenesis by acting on different molecular targets; the downward arrows indicate the down-regulation effects, and the upward arrows indicate the up-regulation of the molecular targets or signaling pathways. Bar-headed lines are used to indicate inhibition.

Graphical abstract legend:

Phytosterols modulate several molecular signaling pathways and cellular mediators that control the survival mechanisms in cancer cells. Phytosterols may promote apoptosis and prevent the proliferation through different pathways that may involve sphingomyelin metabolism, LXR activation, antioxidant mechanisms, and caspases.

References

1. Fitzmaurice, C., Allen, C., Barber, R. M., Barregard, L., Bhutta, Z. A., Brenner, H., Dicker, D. J., Chimed-Orchir, O., Dandona, R., Dandona, L., Fleming, T., Forouzanfar, M. H., Hancock, J., Hay, R. J., Hunter-Merrill, R., Huynh, C., Hosgood, H. D., Johnson, C. O., Jonas, J. B., Khubchandani, J., Kumar, G. A., Kutz, M., Lan, Q., Larson, H. J., Liang, X., Lim, S. S., Lopez, A. D., MacIntyre, M. F., Marczak, L., Marquez, N., Mokdad, A. H., Pinho, C., Pourmalek, F., Salomon, J. A., Sanabria, J. R., Sandar, L., Sartorius, B., Schwartz, S. M., Shackelford, K. A., Shibuya, K., Stanaway, J., Steiner, C., Sun, J., Takahashi, K., Vollset, S. E., Vos, T., Wagner, J. A., Wang, H., Westerman, R., Zeeb, H., Zoeckler, L., Abd-Allah, F., Ahmed, M. B., Alabed, S., Alam, N. K., Aldhahri, S. F., Alem, G., Alemayohu, M. A., Ali, R., Al-Raddadi, R., Amare, A., Amoako, Y., Artaman, A., Asayesh, H., Atnafu, N., Awasthi, A., Saleem, H. B., Barac, A., Bedi, N., Bensenor, I., Berhane, A., Bernabe, E., Betsu, B., Binagwaho, A., Boneya, D., Campos-Nonato, I., Castaneda-Orjuela, C., Catala-Lopez, F., Chiang, P., Chibueze, C., Chitheer, A., Choi, J. Y., Cowie, B., Damtew, S., das Neves, J., Dey, S., Dharmaratne, S., Dhillon, P., Ding, E., Driscoll, T., Ekwueme, D., Endries, A. Y., Farvid, M., Farzadfar, F., Fernandes, J., Fischer, F., TT, G. H., Gebru, A., Gopalani, S., Hailu, A., Horino, M., Horita, N., Husseini, A., Huybrechts, I., Inoue, M., Islami, F., Jakovljevic, M., James, S., Javanbakht, M., Jee, S. H., Kasaeian, A., Kedir, M. S., Khader, Y. S., Khang, Y. H., Kim, D., Leigh, J., Linn, S., Lunevicius, R., El Razek, H. M. A., Malekzadeh, R., Malta, D. C., Marcenes, W., Markos, D., Melaku, Y. A., Meles, K. G., Mendoza, W., Mengiste, D. T., Meretoja, T. J., Miller, T. R., Mohammad, K. A., Mohammadi, A., Mohammed, S., Moradi-Lakeh, M., Nagel, G., Nand, D., Le Nguyen, Q., Nolte, S., Ogbo, F. A., Oladimeji, K. E., Oren, E., Pa, M., Park, E. K., Pereira, D. M., Plass, D., Qorbani, M., Radfar, A., Rafay, A., Rahman, M., Rana, S. M., Soreide, K., Satpathy, M., Sawhney, M., Sepanlou, S. G., Shaikh, M. A., She, J., Shiue, I., Shore, H. R., Shrime, M. G., So, S., Soneji, S., Stathopoulou, V., Stroumpoulis, K., Sufiyan, M. B., Sykes, B. L., Tabares-Seisdedos, R., Tadese, F., Tedla, B. A., Tessema, G. A., Thakur, J. S., Tran, B. X., Ukwaja, K. N., Uzochukwu, B. S. C., Vlassov, V. V., Weiderpass, E., Wubshet Terefe, M., Yebyo, H. G., Yimam, H. H., Yonemoto, N., Younis, M. Z., Yu, C., Zaidi, Z., Zaki, M. E. S., Zenebe, Z. M., Murray, C. J. L., and Naghavi, M. (2017) Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study, *JAMA Oncol 3*, 524-548.
2. (2007) *World Cancer Research Fund - American Institute for Cancer Research. Food, nutrition, physical activity, and the prevention of canbcer: a global perspective*, American Institute for Cancer Research (AICR), Washington DC.
3. Woyengo, T. A., Ramprasath, V. R., and Jones, P. J. (2009) Anticancer effects of phytosterols, *Eur J Clin Nutr 63*, 813-820.
4. Bradford, P. G., and Awad, A. B. (2010) Modulation of signal transduction in cancer cells by phytosterols, *Biofactors 36*, 241-247.
5. Willett, W. C. (2010) Fruits, vegetables, and cancer prevention: turmoil in the produce section, *J Natl Cancer Inst 102*, 510-511.
6. Beliveau, R., and Gingras, D. (2007) Role of nutrition in preventing cancer, *Can Fam Physician 53*, 1905-1911.
7. Barnard, R. J. (2004) Prevention of Cancer Through Lifestyle Changes, *Evid Based Complement Alternat Med 1*, 233-239.
8. Aune, D., Giovannucci, E., Boffetta, P., Fadnes, L. T., Keum, N., Norat, T., Greenwood, D. C., Riboli, E., Vatten, L. J., and Tonstad, S. (2017) Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies, *Int J Epidemiol*.
9. Wang, X., Ouyang, Y., Liu, J., Zhu, M., Zhao, G., Bao, W., and Hu, F. B. (2014) Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies, *BMJ 349*, g4490.
10. Fraser, G. E. (2009) Vegetarian diets: what do we know of their effects on common chronic diseases?, *Am J Clin Nutr 89*, 1607S-1612S.
11. Tantamango-Bartley, Y., Jaceldo-Siegl, K., Fan, J., and Fraser, G. (2013) Vegetarian diets and the incidence of cancer in a low-risk population, *Cancer Epidemiol Biomarkers Prev 22*, 286-294.

12. Giacosa, A., Barale, R., Bavaresco, L., Gatenby, P., Gerbi, V., Janssens, J., Johnston, B., Kas, K., La Vecchia, C., Mainguet, P., Morazzoni, P., Negri, E., Pelucchi, C., Pezzotti, M., and Rondanelli, M. (2013) Cancer prevention in Europe: the Mediterranean diet as a protective choice, *Eur J Cancer Prev 22*, 90-95.
13. Schwingshackl, L., and Hoffmann, G. (2016) Does a Mediterranean-Type Diet Reduce Cancer Risk?, *Curr Nutr Rep 5*, 9-17.
14. Hoffmann, G., and Schwingshackl, L. (2016) Mediterranean diet supplemented with extra virgin olive oil reduces the incidence of invasive breast cancer in a randomised controlled trial, *Evid Based Med 21*, 72.
15. (2003) *Fruits and vegetables*, Vol. 8, International Agency for Research on Cancer, World health Organization, Lyon.
16. Kushi, L. H., Doyle, C., McCullough, M., Rock, C. L., Demark-Wahnefried, W., Bandera, E. V., Gapstur, S., Patel, A. V., Andrews, K., and Gansler, T. (2012) American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity, *CA Cancer J Clin 62*, 30-67.
17. (2015) *Scientific report of the 2015 Dietary Guidelines Advisory Committee (DGAC), .*
18. Butrum, R. R., Clifford, C. K., and Lanza, E. (1988) NCI dietary guidelines: rationale, *Am J Clin Nutr 48*, 888-895.
19. Dewell, A., Weidner, G., Sumner, M. D., Chi, C. S., and Ornish, D. (2008) A very-low-fat vegan diet increases intake of protective dietary factors and decreases intake of pathogenic dietary factors, *J Am Diet Assoc 108*, 347-356.
20. Shibamoto, T., Kanazawa, K., Shahidi, F., and Ho, C. T. (2008) Functional Food and Health: an overview, in *Functional Foods and Health*, pp 1-6, American Chemical Society, Washington.
21. Liu, Y., and Feng, N. (2015) Nanocarriers for the delivery of active ingredients and fractions extracted from natural products used in traditional Chinese medicine (TCM), *Adv Colloid Interface Sci 221*, 60-76.
22. Ramprasath, V. R., and Awad, A. B. (2015) Role of Phytosterols in Cancer Prevention and Treatment, *J AOAC Int 98*, 735-738.
23. Othman, R. A., Myrie, S. B., and Jones, P. J. (2013) Non-cholesterol sterols and cholesterol metabolism in sitosterolemia, *Atherosclerosis 231*, 291-299.
24. (2002) General view of the Scientific Committee on Food on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, with particular attention to the effects of beta-carotene, (Food, S. C. o., Ed.), Brussels.
25. Lagarda, M. J., Garcia-Llatas, G., and Farre, R. (2006) Analysis of phytosterols in foods, *J Pharm Biomed Anal 41*, 1486-1496.
26. Scholz, B., Guth, S., Engel, K. H., and Steinberg, P. (2015) Phytosterol oxidation products in enriched foods: Occurrence, exposure, and biological effects, *Mol Nutr Food Res 59*, 1339-1352.
27. Bradford, P. G., and Awad, A. B. (2007) Phytosterols as anticancer compounds, *Mol Nutr Food Res 51*, 161-170.
28. Luo, X., Su, P., and Zhang, W. (2015) Advances in Microalgae-Derived Phytosterols for Functional Food and Pharmaceutical Applications, *Mar Drugs 13*, 4231-4254.
29. Weihrauch, J. L., and Gardner, J. M. (1978) Sterol content of foods of plant origin, *J Am Diet Assoc 73*, 39-47.
30. Moreau, R. A., Whitaker, B. D., and Hicks, K. B. (2002) Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses, *Prog Lipid Res 41*, 457-500.
31. Jones, P. J., and AbuMweis, S. S. (2009) Phytosterols as functional food ingredients: linkages to cardiovascular disease and cancer, *Curr Opin Clin Nutr Metab Care 12*, 147-151.
32. Racette, S. B., Lin, X., Ma, L., and Ostlund, R. E., Jr. (2015) Natural Dietary Phytosterols, *J AOAC Int 98*, 679-684.
33. Bosner, M. S., Lange, L. G., Stenson, W. F., and Ostlund, R. E., Jr. (1999) Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry, *J Lipid Res 40*, 302-308.
34. Nguyen, T. T. (1999) The cholesterol-lowering action of plant stanol esters, *J Nutr 129*, 2109-2112.
35. Matthan, N. R., and Lichtenstein, A. H. (2004) Approaches to measuring cholesterol absorption in humans, *Atherosclerosis 174*, 197-205.
36. Kudchodkar, B. J., Sodhi, H. S., and Horlick, L. (1973) Absorption of dietary cholesterol in man, *Metabolism 22*, 155-163.

37. Salen, G., Ahrens, E. H., Jr., and Grundy, S. M. (1970) Metabolism of beta-sitosterol in man, *J Clin Invest 49*, 952-967.
38. Ostlund, R. E., Jr. (2007) Phytosterols, cholesterol absorption and healthy diets, *Lipids 42*, 41-45.
39. Heinemann, T., Axtmann, G., and von Bergmann, K. (1993) Comparison of intestinal absorption of cholesterol with different plant sterols in man, *Eur J Clin Invest 23*, 827-831.
40. Ostlund, R. E., Jr., McGill, J. B., Zeng, C. M., Covey, D. F., Stearns, J., Stenson, W. F., and Spilburg, C. A. (2002) Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytosterols and phytostanols in humans, *Am J Physiol Endocrinol Metab 282*, E911-916.
41. Chen, B., McClements, D. J., and Decker, E. A. (2013) Design of foods with bioactive lipids for improved health, *Annu Rev Food Sci Technol 4*, 35-56.
42. Saura-Calixto, F., and Goni, I. (2009) Definition of the Mediterranean diet based on bioactive compounds, *Crit Rev Food Sci Nutr 49*, 145-152.
43. Lin, Y., Knol, D., Menendez-Carreno, M., Baris, R., Janssen, H. G., and Trautwein, E. A. (2018) Oxidation of sitosterol and campesterol in foods upon cooking with liquid margarines without and with added plant sterol esters, *Food Chem 241*, 387-396.
44. Lin, Y., Knol, D., and Trautwein, E. A. (2016) Phytosterol oxidation products (POP) in foods with added phytosterols and estimation of their daily intake: A literature review, *Eur J Lipid Sci Technol 118*, 1423-1438.
45. Mouhid, L., Corzo-Martinez, M., Torres, C., Vazquez, L., Reglero, G., Fornari, T., and Ramirez de Molina, A. (2017) Improving In Vivo Efficacy of Bioactive Molecules: An Overview of Potentially Antitumor Phytochemicals and Currently Available Lipid-Based Delivery Systems, *J Oncol 2017*, 7351976.
46. Imanaka, H., Koide, H., Shimizu, K., Asai, T., Kinouchi Shimizu, N., Ishikado, A., Makino, T., and Oku, N. (2008) Chemoprevention of tumor metastasis by liposomal beta-sitosterol intake, *Biol Pharm Bull 31*, 400-404.
47. Plat, J., Mackay, D., Baumgartner, S., Clifton, P. M., Gylling, H., and Jones, P. J. (2012) Progress and prospective of plant sterol and plant stanol research: report of the Maastricht meeting, *Atherosclerosis 225*, 521-533.
48. Soupas, L., Juntunen, L., Lampi, A. M., and Piironen, V. (2004) Effects of sterol structure, temperature, and lipid medium on phytosterol oxidation, *J Agric Food Chem 52*, 6485-6491.
49. Lutjohann, D., Meese, C. O., Crouse, J. R., 3rd, and von Bergmann, K. (1993) Evaluation of deuterated cholesterol and deuterated sitostanol for measurement of cholesterol absorption in humans, *J Lipid Res 34*, 1039-1046.
50. Patel, M. D., and Thompson, P. D. (2006) Phytosterols and vascular disease, *Atherosclerosis 186*, 12-19.
51. Reddy, B. S., Martin, C. W., and Wynder, E. L. (1977) Fecal bile acids and cholesterol metabolites of patients with ulcerative colitis, a high-risk group for development of colon cancer, *Cancer Res 37*, 1697-1701.
52. Reddy, B. S., and Watanabe, K. (1979) Effect of cholesterol metabolites and promoting effect of lithocholic acid in colon carcinogenesis in germ-free and conventional F344 rats, *Cancer Res 39*, 1521-1524.
53. Reddy, B. S., and Wynder, E. L. (1977) Metabolic epidemiology of colon cancer. Fecal bile acids and neutral sterols in colon cancer patients and patients with adenomatous polyps, *Cancer 39*, 2533-2539.
54. Plat, J., and Mensink, R. P. (2001) Effects of diets enriched with two different plant stanol ester mixtures on plasma ubiquinol-10 and fat-soluble antioxidant concentrations, *Metabolism 50*, 520-529.
55. Katan, M. B., Grundy, S. M., Jones, P., Law, M., Miettinen, T., and Paoletti, R. (2003) Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels, *Mayo Clin Proc 78*, 965-978.
56. Baumgartner, S., Ras, R. T., Trautwein, E. A., Mensink, R. P., and Plat, J. (2017) Plasma fat-soluble vitamin and carotenoid concentrations after plant sterol and plant stanol consumption: a meta-analysis of randomized controlled trials, *Eur J Nutr 56*, 909-923.
57. Norum, K. R., and Blomhoff, R. (1992) McCollum Award Lecture, 1992: vitamin A absorption, transport, cellular uptake, and storage, *Am J Clin Nutr 56*, 735-744.
58. Parker, R. S. (1996) Absorption, metabolism, and transport of carotenoids, *FASEB J 10*, 542-551.
59. Nair, P. P., Turjman, N., Kessie, G., Calkins, B., Goodman, G. T., Davidovitz, H., and Nimmagadda, G. (1984) Diet, nutrition intake, and metabolism in populations at high and low

risk for colon cancer. Dietary cholesterol, beta-sitosterol, and stigmasterol, *Am J Clin Nutr 40*, 927-930.
60. Sanclemente, T., Marques-Lopes, I., Fajo-Pascual, M., Cofan, M., Jarauta, E., Ros, E., Puzo, J., and Garcia-Otin, A. L. (2009) A moderate intake of phytosterols from habitual diet affects cholesterol metabolism, *J Physiol Biochem 65*, 397-404.
61. Toledo, E., Salas-Salvado, J., Donat-Vargas, C., Buil-Cosiales, P., Estruch, R., Ros, E., Corella, D., Fito, M., Hu, F. B., Aros, F., Gomez-Gracia, E., Romaguera, D., Ortega-Calvo, M., Serra-Majem, L., Pinto, X., Schroder, H., Basora, J., Sorli, J. V., Bullo, M., Serra-Mir, M., and Martinez-Gonzalez, M. A. (2015) Mediterranean Diet and Invasive Breast Cancer Risk Among Women at High Cardiovascular Risk in the PREDIMED Trial: A Randomized Clinical Trial, *JAMA Intern Med 175*, 1752-1760.
62. Grosso, G., Buscemi, S., Galvano, F., Mistretta, A., Marventano, S., La Vela, V., Drago, F., Gangi, S., Basile, F., and Biondi, A. (2013) Mediterranean diet and cancer: epidemiological evidence and mechanism of selected aspects, *BMC Surg 13 Suppl 2*, S14.
63. Bin Sayeed, M. S., Karim, S. M. R., Sharmin, T., and Morshed, M. M. (2016) Critical Analysis on Characterization, Systemic Effect, and Therapeutic Potential of Beta-Sitosterol: A Plant-Derived Orphan Phytosterol, *Medicines (Basel) 3*.
64. Llaverias, G., Escola-Gil, J. C., Lerma, E., Julve, J., Pons, C., Cabre, A., Cofan, M., Ros, E., Sanchez-Quesada, J. L., and Blanco-Vaca, F. (2013) Phytosterols inhibit the tumor growth and lipoprotein oxidizability induced by a high-fat diet in mice with inherited breast cancer, *J Nutr Biochem 24*, 39-48.
65. Shenouda, N. S., Sakla, M. S., Newton, L. G., Besch-Williford, C., Greenberg, N. M., MacDonald, R. S., and Lubahn, D. B. (2007) Phytosterol Pygeum africanum regulates prostate cancer in vitro and in vivo, *Endocrine 31*, 72-81.
66. Almazari, I., and Surh, Y. (2012) Cancer Chemopreventive and Therapeutic Potential of Guggulsterone., in *Natural Products in Cancer Prevention and Therapy. Topics in Current Chemistry.* (Pezzuto, J., and Suh, N., Eds.), Springer, Berlin, Heidelberg.
67. Sook, S. H., Lee, H. J., Kim, J. H., Sohn, E. J., Jung, J. H., Kim, B., Jeong, S. J., and Kim, S. H. (2014) Reactive oxygen species-mediated activation of AMP-activated protein kinase and c-Jun N-terminal kinase plays a critical role in beta-sitosterol-induced apoptosis in multiple myeloma U266 cells, *Phytother Res 28*, 387-394.
68. Zhao, Y., Chang, S. K., Qu, G., Li, T., and Cui, H. (2009) Beta-sitosterol inhibits cell growth and induces apoptosis in SGC-7901 human stomach cancer cells, *J Agric Food Chem 57*, 5211-5218.
69. Moon, D. O., Kim, M. O., Choi, Y. H., and Kim, G. Y. (2008) beta-Sitosterol induces G2/M arrest, endoreduplication, and apoptosis through the Bcl-2 and PI3K/Akt signaling pathways, *Cancer Lett 264*, 181-191.
70. Park, C., Moon, D. O., Rhu, C. H., Choi, B. T., Lee, W. H., Kim, G. Y., and Choi, Y. H. (2007) Beta-sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio, *Biol Pharm Bull 30*, 1317-1323.
71. Awad, A. B., Downie, A. C., and Fink, C. S. (2000) Inhibition of growth and stimulation of apoptosis by beta-sitosterol treatment of MDA-MB-231 human breast cancer cells in culture, *Int J Mol Med 5*, 541-545.
72. Rubis, B., Polrolniczak, A., Knula, H., Potapinska, O., Kaczmarek, M., and Rybczynska, M. (2010) Phytosterols in physiological concentrations target multidrug resistant cancer cells, *Med Chem 6*, 184-190.
73. Sharmila, R., and Sindhu, G. (2017) Modulation of Angiogenesis, Proliferative Response and Apoptosis by beta-Sitosterol in Rat Model of Renal Carcinogenesis, *Indian J Clin Biochem 32*, 142-152.
74. Park, C., Moon, D. O., Ryu, C. H., Choi, B., Lee, W., Kim, G. Y., and Choi, Y. (2008) Beta-sitosterol sensitizes MDA-MB-231 cells to TRAIL-induced apoptosis, *Acta Pharmacol Sin 29*, 341-348.
75. Moon, D. O., Lee, K. J., Choi, Y. H., and Kim, G. Y. (2007) Beta-sitosterol-induced-apoptosis is mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells, *Int Immunopharmacol 7*, 1044-1053.
76. Choi, Y. H., Kong, K. R., Kim, Y. A., Jung, K. O., Kil, J. H., Rhee, S. H., and Park, K. Y. (2003) Induction of Bax and activation of caspases during beta-sitosterol-mediated apoptosis in human colon cancer cells, *Int J Oncol 23*, 1657-1662.
77. Awad, A. B., Roy, R., and Fink, C. S. (2003) Beta-sitosterol, a plant sterol, induces apoptosis and activates key caspases in MDA-MB-231 human breast cancer cells, *Oncol Rep 10*, 497-500.

78. von Holtz, R. L., Fink, C. S., and Awad, A. B. (1998) beta-Sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells, *Nutr Cancer 32*, 8-12.
79. Awad, A. B., Chinnam, M., Fink, C. S., and Bradford, P. G. (2007) beta-Sitosterol activates Fas signaling in human breast cancer cells, *Phytomedicine 14*, 747-754.
80. Smyth, M. J., Perry, D. K., Zhang, J., Poirier, G. G., Hannun, Y. A., and Obeid, L. M. (1996) prICE: a downstream target for ceramide-induced apoptosis and for the inhibitory action of Bcl-2, *Biochem J 316 (Pt 1)*, 25-28.
81. von Haefen, C., Wieder, T., Gillissen, B., Starck, L., Graupner, V., Dorken, B., and Daniel, P. T. (2002) Ceramide induces mitochondrial activation and apoptosis via a Bax-dependent pathway in human carcinoma cells, *Oncogene 21*, 4009-4019.
82. Tasyriq, M., Najmuldeen, I. A., In, L. L., Mohamad, K., Awang, K., and Hasima, N. (2012) 7alpha-Hydroxy-beta-Sitosterol from Chisocheton tomentosus Induces Apoptosis via Dysregulation of Cellular Bax/Bcl-2 Ratio and Cell Cycle Arrest by Downregulating ERK1/2 Activation, *Evid Based Complement Alternat Med 2012*, 765316.
83. Shi, J. J., Jia, X. L., Li, M., Yang, N., Li, Y. P., Zhang, X., Gao, N., and Dang, S. S. (2015) Guggulsterone induces apoptosis of human hepatocellular carcinoma cells through intrinsic mitochondrial pathway, *World J Gastroenterol 21*, 13277-13287.
84. Zhong, F., Yang, J., Tong, Z. T., Chen, L. L., Fan, L. L., Wang, F., Zha, X. L., and Li, J. (2015) Guggulsterone inhibits human cholangiocarcinoma Sk-ChA-1 and Mz-ChA-1 cell growth by inducing caspase-dependent apoptosis and downregulation of survivin and Bcl-2 expression, *Oncol Lett 10*, 1416-1422.
85. Guan, X. (2015) Cancer metastases: challenges and opportunities, *Acta Pharm Sin B 5*, 402-418.
86. Awad, A. B., Fink, C. S., Williams, H., and Kim, U. (2001) In vitro and in vivo (SCID mice) effects of phytosterols on the growth and dissemination of human prostate cancer PC-3 cells, *Eur J Cancer Prev 10*, 507-513.
87. Awad, A. B., Williams, H., and Fink, C. S. (2001) Phytosterols reduce in vitro metastatic ability of MDA-MB-231 human breast cancer cells, *Nutr Cancer 40*, 157-164.
88. Al-Husein, B., Abdalla, M., Trepte, M., Deremer, D. L., and Somanath, P. R. (2012) Antiangiogenic therapy for cancer: an update, *Pharmacotherapy 32*, 1095-1111.
89. Masson, N., and Ratcliffe, P. J. (2014) Hypoxia signaling pathways in cancer metabolism: the importance of co-selecting interconnected physiological pathways, *Cancer Metab 2*, 3.
90. Moon, E. J., Lee, Y. M., Lee, O. H., Lee, M. J., Lee, S. K., Chung, M. H., Park, Y. I., Sung, C. K., Choi, J. S., and Kim, K. W. (1999) A novel angiogenic factor derived from Aloe vera gel: beta-sitosterol, a plant sterol, *Angiogenesis 3*, 117-123.
91. Choi, S., Kim, K. W., Choi, J. S., Han, S. T., Park, Y. I., Lee, S. K., Kim, J. S., and Chung, M. H. (2002) Angiogenic activity of beta-sitosterol in the ischaemia/reperfusion-damaged brain of Mongolian gerbil, *Planta Med 68*, 330-335.
92. Choi, J. M., Lee, E. O., Lee, H. J., Kim, K. H., Ahn, K. S., Shim, B. S., Kim, N. I., Song, M. C., Baek, N. I., and Kim, S. H. (2007) Identification of campesterol from Chrysanthemum coronarium L. and its antiangiogenic activities, *Phytother Res 21*, 954-959.
93. Shuman Moss, L. A., Jensen-Taubman, S., Rubinstein, D., Viole, G., and Stetler-Stevenson, W. G. (2014) Dietary intake of a plant phospholipid/lipid conjugate reduces lung cancer growth and tumor angiogenesis, *Carcinogenesis 35*, 1556-1563.
94. Goraca, A., Huk-Kolega, H., Piechota, A., Kleniewska, P., Ciejka, E., and Skibska, B. (2011) Lipoic acid - biological activity and therapeutic potential, *Pharmacol Rep 63*, 849-858.
95. Jayaprakasam, B., Vanisree, M., Zhang, Y., Dewitt, D. L., and Nair, M. G. (2006) Impact of alkyl esters of caffeic and ferulic acids on tumor cell proliferation, cyclooxygenase enzyme, and lipid peroxidation, *J Agric Food Chem 54*, 5375-5381.
96. Bouic, P. J., Etsebeth, S., Liebenberg, R. W., Albrecht, C. F., Pegel, K., and Van Jaarsveld, P. P. (1996) beta-Sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination, *Int J Immunopharmacol 18*, 693-700.
97. Bouic, P. J. (2001) The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years, *Curr Opin Clin Nutr Metab Care 4*, 471-475.
98. Li, Y. C., Park, M. J., Ye, S. K., Kim, C. W., and Kim, Y. N. (2006) Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents, *Am J Pathol 168*, 1107-1118; quiz 1404-1105.
99. Li, C., Yang, L., Zhang, D., and Jiang, W. (2016) Systematic review and meta-analysis suggest that dietary cholesterol intake increases risk of breast cancer, *Nutr Res 36*, 627-635.

100. Hu, J., La Vecchia, C., de Groh, M., Negri, E., Morrison, H., and Mery, L. (2012) Dietary cholesterol intake and cancer, *Ann Oncol 23*, 491-500.
101. Pelton, K., Coticchia, C. M., Curatolo, A. S., Schaffner, C. P., Zurakowski, D., Solomon, K. R., and Moses, M. A. (2014) Hypercholesterolemia induces angiogenesis and accelerates growth of breast tumors in vivo, *Am J Pathol 184*, 2099-2110.
102. Papanagnou, P., Stivarou, T., Papageorgiou, I., Papadopoulos, G. E., and Pappas, A. (2017) Marketed drugs used for the management of hypercholesterolemia as anticancer armament, *Onco Targets Ther 10*, 4393-4411.
103. Nielsen, S. F., Nordestgaard, B. G., and Bojesen, S. E. (2012) Statin use and reduced cancer-related mortality, *N Engl J Med 367*, 1792-1802.
104. Yokomichi, H., Nagai, A., Hirata, M., Tamakoshi, A., Kiyohara, Y., Kamatani, Y., Muto, K., Ninomiya, T., Matsuda, K., Kubo, M., Nakamura, Y., and Yamagata, Z. (2017) Statin use and all-cause and cancer mortality: BioBank Japan cohort, *J Epidemiol 27*, S84-S91.
105. Mei, Z., Liang, M., Li, L., Zhang, Y., Wang, Q., and Yang, W. (2017) Effects of statins on cancer mortality and progression: A systematic review and meta-analysis of 95 cohorts including 1,111,407 individuals, *Int J Cancer 140*, 1068-1081.
106. Lin, C. Y., and Gustafsson, J. A. (2015) Targeting liver X receptors in cancer therapeutics, *Nat Rev Cancer 15*, 216-224.
107. Hovenkamp, E., Demonty, I., Plat, J., Lutjohann, D., Mensink, R. P., and Trautwein, E. A. (2008) Biological effects of oxidized phytosterols: a review of the current knowledge, *Prog Lipid Res 47*, 37-49.
108. Hong, C., and Tontonoz, P. (2014) Liver X receptors in lipid metabolism: opportunities for drug discovery, *Nat Rev Drug Discov 13*, 433-444.
109. Vedin, L. L., Lewandowski, S. A., Parini, P., Gustafsson, J. A., and Steffensen, K. R. (2009) The oxysterol receptor LXR inhibits proliferation of human breast cancer cells, *Carcinogenesis 30*, 575-579.
110. Lo Sasso, G., Bovenga, F., Murzilli, S., Salvatore, L., Di Tullio, G., Martelli, N., D'Orazio, A., Rainaldi, S., Vacca, M., Mangia, A., Palasciano, G., and Moschetta, A. (2013) Liver X receptors inhibit proliferation of human colorectal cancer cells and growth of intestinal tumors in mice, *Gastroenterology 144*, 1497-1507, 1507 e1491-1413.
111. Vigushin, D. M., Dong, Y., Inman, L., Peyvandi, N., Alao, J. P., Sun, C., Ali, S., Niesor, E. J., Bentzen, C. L., and Coombes, R. C. (2004) The nuclear oxysterol receptor LXRalpha is expressed in the normal human breast and in breast cancer, *Med Oncol 21*, 123-131.
112. Chuu, C. P., Kokontis, J. M., Hiipakka, R. A., and Liao, S. (2007) Modulation of liver X receptor signaling as novel therapy for prostate cancer, *J Biomed Sci 14*, 543-553.
113. Fukuchi, J., Kokontis, J. M., Hiipakka, R. A., Chuu, C. P., and Liao, S. (2004) Antiproliferative effect of liver X receptor agonists on LNCaP human prostate cancer cells, *Cancer Res 64*, 7686-7689.
114. Zhu, Y., Soroka, D., and Sang, S. (2015) Oxyphytosterols as active ingredients in wheat bran suppress human colon cancer cell growth: identification, chemical synthesis, and biological evaluation, *J Agric Food Chem 63*, 2264-2276.
115. Grandgirard, A., Martine, L., Demaison, L., Cordelet, C., Joffre, C., Berdeaux, O., and Semon, E. (2004) Oxyphytosterols are present in plasma of healthy human subjects, *Br J Nutr 91*, 101-106.
116. Awad, A. B., Chen, Y. C., Fink, C. S., and Hennessey, T. (1996) beta-Sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids, *Anticancer Res 16*, 2797-2804.
117. Awad, A. B., von Holtz, R. L., Cone, J. P., Fink, C. S., and Chen, Y. C. (1998) beta-Sitosterol inhibits growth of HT-29 human colon cancer cells by activating the sphingomyelin cycle, *Anticancer Res 18*, 471-473.
118. Hannun, Y. A., Luberto, C., and Argraves, K. M. (2001) Enzymes of sphingolipid metabolism: from modular to integrative signaling, *Biochemistry 40*, 4893-4903.
119. Mathias, S., Pena, L. A., and Kolesnick, R. N. (1998) Signal transduction of stress via ceramide, *Biochem J 335 (Pt 3)*, 465-480.
120. Gorrini, C., Harris, I. S., and Mak, T. W. (2013) Modulation of oxidative stress as an anticancer strategy, *Nat Rev Drug Discov 12*, 931-947.
121. Vivancos, M., and Moreno, J. J. (2005) beta-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages, *Free Radic Biol Med 39*, 91-97.
122. Moreno, J. J. (2003) Effect of olive oil minor components on oxidative stress and arachidonic acid mobilization and metabolism by macrophages RAW 264.7, *Free Radic Biol Med 35*, 1073-1081.

123. Baskar, A. A., Al Numair, K. S., Gabriel Paulraj, M., Alsaif, M. A., Muamar, M. A., and Ignacimuthu, S. (2012) beta-sitosterol prevents lipid peroxidation and improves antioxidant status and histoarchitecture in rats with 1,2-dimethylhydrazine-induced colon cancer, *J Med Food 15*, 335-343.
124. Grattan, B. J., Jr. (2013) Plant sterols as anticancer nutrients: evidence for their role in breast cancer, *Nutrients 5*, 359-387.
125. Shi, C., Wu, F., Zhu, X. C., and Xu, J. (2013) Incorporation of beta-sitosterol into the membrane increases resistance to oxidative stress and lipid peroxidation via estrogen receptor-mediated PI3K/GSK3beta signaling, *Biochim Biophys Acta 1830*, 2538-2544.
126. Hirsch, H. A., Iliopoulos, D., Joshi, A., Zhang, Y., Jaeger, S. A., Bulyk, M., Tsichlis, P. N., Shirley Liu, X., and Struhl, K. (2010) A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases, *Cancer Cell 17*, 348-361.
127. Gonzalez-Chavarria, I., Cerro, R. P., Parra, N. P., Sandoval, F. A., Zuniga, F. A., Omazabal, V. A., Lamperti, L. I., Jimenez, S. P., Fernandez, E. A., Gutierrez, N. A., Rodriguez, F. S., Onate, S. A., Sanchez, O., Vera, J. C., and Toledo, J. R. (2014) Lectin-like oxidized LDL receptor-1 is an enhancer of tumor angiogenesis in human prostate cancer cells, *PLoS One 9*, e106219.
128. Fantozzi, A., and Christofori, G. (2006) Mouse models of breast cancer metastasis, *Breast Cancer Res 8*, 212.
129. Aviram, M., Dankner, G., Cogan, U., Hochgraf, E., and Brook, J. G. (1992) Lovastatin inhibits low-density lipoprotein oxidation and alters its fluidity and uptake by macrophages: in vitro and in vivo studies, *Metabolism 41*, 229-235.
130. Ritschel, W. A., Kastner, U., Hussain, A. S., and Koch, H. P. (1990) Pharmacokinetics and bioavailability of beta-sitosterol in the beagle dog, *Arzneimittelforschung 40*, 463-468.
131. Awad, A. B., Barta, S. L., Fink, C. S., and Bradford, P. G. (2008) beta-Sitosterol enhances tamoxifen effectiveness on breast cancer cells by affecting ceramide metabolism, *Mol Nutr Food Res 52*, 419-426.
132. Daly, T. J., Aherne, S. A., O'Connor, T. P., and O'Brien, N. M. (2009) Lack of genoprotective effect of phytosterols and conjugated linoleic acids on Caco-2 cells, *Food Chem Toxicol 47*, 1791-1796.
133. Maguire, L., Konoplyannikov, M., Ford, A., Maguire, A. R., and O'Brien, N. M. (2003) Comparison of the cytotoxic effects of beta-sitosterol oxides and a cholesterol oxide, 7beta-hydroxycholesterol, in cultured mammalian cells, *Br J Nutr 90*, 767-775.
134. Mendilaharsu, M., De Stefani, E., Deneo-Pellegrini, H., Carzoglio, J., and Ronco, A. (1998) Phytosterols and risk of lung cancer: a case-control study in Uruguay, *Lung Cancer 21*, 37-45.
135. Ronco, A., De Stefani, E., Boffetta, P., Deneo-Pellegrini, H., Mendilaharsu, M., and Leborgne, F. (1999) Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay, *Nutr Cancer 35*, 111-119.
136. De Stefani, E., Brennan, P., Boffetta, P., Ronco, A. L., Mendilaharsu, M., and Deneo-Pellegrini, H. (2000) Vegetables, fruits, related dietary antioxidants, and risk of squamous cell carcinoma of the esophagus: a case-control study in Uruguay, *Nutr Cancer 38*, 23-29.
137. De Stefani, E., Boffetta, P., Ronco, A. L., Brennan, P., Deneo-Pellegrini, H., Carzoglio, J. C., and Mendilaharsu, M. (2000) Plant sterols and risk of stomach cancer: a case-control study in Uruguay, *Nutr Cancer 37*, 140-144.
138. McCann, S. E., Freudenheim, J. L., Marshall, J. R., Brasure, J. R., Swanson, M. K., and Graham, S. (2000) Diet in the epidemiology of endometrial cancer in western New York (United States), *Cancer Causes Control 11*, 965-974.
139. McCann, S. E., Freudenheim, J. L., Marshall, J. R., and Graham, S. (2003) Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups, *J Nutr 133*, 1937-1942.
140. Huang, J., Xu, M., Fang, Y. J., Lu, M. S., Pan, Z. Z., Huang, W. Q., Chen, Y. M., and Zhang, C. X. (2017) Association between phytosterol intake and colorectal cancer risk: a case-control study, *Br J Nutr 117*, 839-850.
141. Normen, A. L., Brants, H. A., Voorrips, L. E., Andersson, H. A., van den Brandt, P. A., and Goldbohm, R. A. (2001) Plant sterol intakes and colorectal cancer risk in the Netherlands Cohort Study on Diet and Cancer, *Am J Clin Nutr 74*, 141-148.
142. Strom, S. S., Yamamura, Y., Duphorne, C. M., Spitz, M. R., Babaian, R. J., Pillow, P. C., and Hursting, S. D. (1999) Phytoestrogen intake and prostate cancer: a case-control study using a new database, *Nutr Cancer 33*, 20-25.
143. Long, S., Sousa, E., Kijjoa, A., and Pinto, M. M. (2016) Marine Natural Products as Models to Circumvent Multidrug Resistance, *Molecules 21*.