Role of Phytoestrogens in Cancer Therapy

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Abstract

Cancer is a leading cause of death worldwide, and the numbers of new cancer cases are expected to continue to rise. The main goals of cancer therapy include removing the primary tumor, preventing the spread of distant metastases, and improving survival and quality of life for the patients. To attain these goals of cancer therapy, the combination of different chemotherapeutics, as opposed to the conventional single-agent treatment, is an emerging area of research. Given the potential risks of drug toxicity in such treatment, the focus is to have a second compound that increases the anticancer potential of the primary agent but which reduces toxicity. There is an ever growing interest in treatment with natural compounds, such as plant phytoestrogens, as an adjuvant cancer therapy along with conventional cancer therapy. The question remains whether or not adding these compounds to the cancer therapy regimen as a second agent would be beneficial, and if they are safe to be used among cancer patients. The current literature suggests that phytoestrogen treatment is capable of inducing G2/M cell cycle arrest in a number of cancer cell lines, as well as upregulating cell cycle inhibitory molecules. Phytoestrogen therapy has been shown to inhibit inflammation, angiogenesis and metastases in various *in vivo* tumor models, and pronounced benefits have been observed when combined with radiation therapy. The lack of side effects from phase I and II clinical trials of phytoestrogens in cancer therapy points towards their safety, but to further understand their added benefit clinical studies with large sample sizes are required. We have reviewed the recent research studies in these areas in an attempt to find evidence for their role in cancer therapy as well as safety.

Abbreviations

CAM:	chicken egg chorioallantoic membrane
COX-2:	cyclooxygenase-2
Cdk:	cyclin-dependent kinase
ELN:	enterolactone
HMR:	hydroxymatairesinol
NSAID:	nonsteroidal anti-inflammatory drugs
NFκB:	nuclear factor kappa B
OPN:	osteopontin
PSA:	prostate specific antigen
SOD1:	superoxide dismutase-1
TPA:	12-*O*-tetradecanoylphorbol 13-acetate
VEGF:	vascular growth factor

Introduction

In many patients with breast cancer and prostate cancer, the tumors are hormonally dependent [1, 2]. This has led to anticancer therapeutic approaches that involve antagonists to estrogens (the use of tamoxifen in breast cancer) [3], or to inhibitors of estrogen/androgen synthesis (aromatase inhibitors in breast cancer [4] and diethylstilbestrol and finasteride in prostate cancer [5]. Peri- and postmenopausal women have also been treated with estrogens to prevent bone and cognition loss and to improve cardiovascular risk [6]. The Women's Health Trial (randomized, placebo-controlled clinical trial) revealed that, contrary to expectation, the presumed benefit of the estrogens on cardiovascular risk was minimal or absent [7]. This created a concern that the estrogen therapy, while still beneficial regarding bone loss, increases breast cancer risk. The latter has been

estimated as being a 1–2% increase in risk per year of use of estrogen therapy [8]. This has promoted the search for selective estrogen receptor modulators (SERMs) – compounds that provide benefit by promoting good bone health, but which do not act as estrogens on estrogen-sensitive tumors [9]. Raloxifene is an example of an SERM that was generated by chemical synthesis [10]. However, investigators have also pursued the concept that plant estrogens (phytoestrogens) that are part of the diet may have a role as natural SERMs [10, 11].
Besides their property of being estrogen receptor agonists, phytoestrogens have several other biological mechanisms of action. They include being protein tyrosine kinase inhibitors (e.g., the epidermal growth factor receptor) [12], antioxidants [13], inhibitors of tumor necrosis factor alpha [14], inhibitors of 3*β*- and 17*β*-hydroxysteroid dehydrogenase activities [15, 16] and inhibitors of aromatase mRNA expression and activity [16], and agonists of the peroxisome proliferator activated-receptor alpha and gamma (PPAR) [17, 18]. These other properties would be expected to differentiate phytoestrogens from physiological estrogens. Indeed, they have been reported to inhibit cell cycle events and prevent cancer in different preclinical models. In addition, epidemiological data have revealed that populations with a high intake of phytoestrogens in the diet have a very much lower incidence of breast and prostate cancer [19, 20]. Since they can be tolerated at oral doses 16 times the maximum intake from the diet [21, 22], clinical investigations are underway to evaluate the effectiveness of phytoestrogens in the treatment of breast and prostate cancers. The present review will focus on roles of phytoestrogens in cell cycle regulation, inflammation, metastasis, and survival as they relate to the ongoing clinical trials. The literature search was performed with the PubMed search engine using the following criteria: manuscript publication years 2002–2009, and combinations of search terms: phytoestrogens, soy isoflavones, cancer, mechanism of action in cultured cell lines, various animal models, cancer therapy, survivorship and clinical experiments. If the manuscripts were not immediately available, reprints were requested from the corresponding authors. The principal exclusion criterion was for those manuscripts that added little or no new data to the existing information. In such cases, the original manuscripts were used for this study – this expanded our search time span beyond the initial 7–8 years guideline. The current ongoing trials on phytoestrogens were searched from the National Institutes of Health website to attain the most current information on such studies.

Chemistry and Dietary Forms of Phytoestrogens

Phytoestrogens are members of the polyphenols that are widely distributed throughout the plant kingdom. They are a subclass of the polyphenols that have structural similarity to the endogenous hormone 17*β*-estradiol, and bind to estrogen receptors. They may be broadly classified into four classes – isoflavones, lignans, coumestans and resorcyclic acid lactones or myco-estrogens. As their name suggests, the latter are a class of phytoestrogens that are produced by mold found on cereal crops. This review will address isoflavones (**Fig. 1**) and lignans (**Fig. 2**), as most research is focused on these two classes and very little is known about the other two classes. The main dietary sources of isoflavones include legumes such as soybean, kala channa, mung bean (green lentil), red lentils and red clover. Daidzein and genistein are the two major isoflavones present in soy. Lignans are

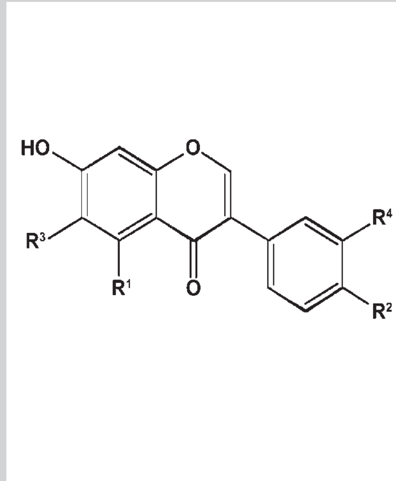

Fig. 1 Chemical structures of isoflavones. Daidzein (R^1 = H, R^2 = OH, R^3 = H, R^4 = H), genistein (R^1 = OH, R^2 = OH, R^3 = H, R^4 = H), glycitein (R^1 = H, R^2 = OH, R^3 = OCH_3, R^4 = H), factor 2 (R^1 = H, R^2 = OH, R^3 = OH, R^4 = H), formononetin (R^1 = H, R^2 = OCH_3, R^3 = H, R^4 = H), biochanin A (R^1 = OH, R^2 = OCH_3, R^3 = H, R^4 = H), orobol (R^1 = OH, R^2 = OH, R^3 = H, R^4 = OH).

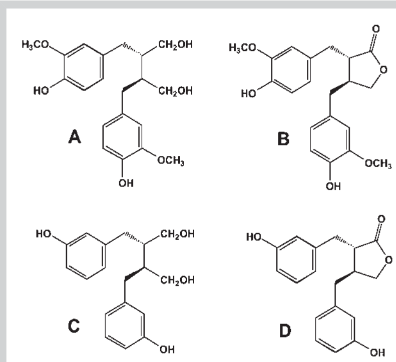

Fig. 2 Chemical structures of lignans. Plant lignans secoisolariciresinol (**A**) and matairesinol (**B**) and mammalian lignans enterodiol (**C**) and enterolactone (**D**).

present mainly in flaxseed, seaweed, whole grains, legumes, oil seeds, fruits and vegetables. The two main lignans in food include secoisolariciresinol diglucoside and matairesinol, which are also the precursors of the mammalian lignans enterodiol and enterolactone. The phytoestrogen content of grain/cereal crops may be affected by genetic, environmental factors, as well as quantification methods used [23, 24]. Intestinal bacteria play an essential role in the absorption and metabolism of both isoflavones and lignans. The detailed background on absorption and metabolism of different isoflavones and lignans is beyond the scope of this review – see [25–27]. For a detailed list of the isoflavone content of food items, please see the United States Department of Agriculture online database [28].

Doses and Metabolism of Phytoestrogens

In evaluating the potential uses(s) of phytoestrogens, careful consideration should be given to the doses or concentrations used in preclinical experiments (cultured cell lines and animal models) and in clinical trials. When isoflavones are administered orally, intestinal cells are exposed to high phytoestrogen concentrations (20–100 µM). Although the isoflavones are well absorbed in the small intestine [29], they are re-excreted into bile and hence the intestine as *β*-glucuronides. These are not well absorbed from the intestine until bacterial *β*-glucuronidases hydrolyze them in the large bowel. Cell culture experiments using intestinal cancer cells at high phytoestrogen concentrations are therefore relevant to *in* *vivo* situations. In contrast, cancer cells in organs at peripheral sites are exposed to phytoestrogen concentrations that are less than 1 µM, most of which are inactive *β*-glucuronide conjugates. When phytoestrogens are administered intravenously or intraperitoneally, the unconjugated (active) phytoestrogens can persist for longer periods of time and at higher concentrations. Phytoestrogen concentrations are nonetheless high (1–50 µM depending on dose) in two other non-gastrointestinal body fluids, in the urine [30] and prostatic fluid [31]. Since rodents have a high rate of metabolism, typically tenfold higher oral doses are required to achieve plasma phytoestrogen concentrations comparable to those in humans. Therefore, a daily dose of 50–100 mg isoflavones (0.7–1.4 mg/kg body weight) in humans would be equivalent to a daily dose of 7–14 mg/kg body weight in rodent models. Also note that giving a rat 1 mg of genistein per day is equivalent to ~ 4 mg/kg body weight (assuming a mean body weight of 250 g). In the mouse a 1 mg dose of genistein is equivalent to 25–50 mg/kg body weight (for mouse body weights of 20–40 g).

Effects of Phytoestrogens on the Cell Cycle

The cell cycle consists of four phases – G1, S, G2 and M. The progression through each phase is both ordered and controlled by various regulatory signaling molecules, and disruption in the regulation can result in neoplastic growth or cancer [32]. There are specific complexes for each phase and formation of cyclins and cyclin-dependent kinase (cdk) complexes and their degradation is essential for cell cycle progression. A number of studies have described an important role of phytoestrogens in regulating the cell cycle (**Table 1**). In a highly metastatic bladder cancer cell line (253J B–V), genistein has been shown to inhibit cell growth by inducing cell cycle arrest at the G2/M transition, and significantly reduced the expression of cell cycle regulators cyclin B1 and Cdk-1 [33]. The similar effect of genistein on G2/M arrest was observed in the cervical cancer cell line ME180 that contains integrated HPV-16 and HPV-18 [34]. In prostate cancer PC-3 cells, treatment with genistein, radiation, or as a combined treatment showed G2/M phase cell cycle arrest, and increased apoptosis in the combined treatment [35]. To understand the influence of the combined treatment on cell cycle progression, Western blot analysis of cell cycle regulatory molecule expression revealed a significant downregulation of cyclin B1 and upregulation of inhibitory molecule P21^{WAF1} for genistein and the combined treatment. The monotherapy with either radiation or genistein increased the P21^{WAF1} expression. However, radiation compared to all other treatments significantly increased cyclin B1 protein, suggesting that the combination treatment is needed to achieve optimum benefit than either treatment alone [12]. Soy isoflavones have growth inhibitory effects on breast, prostate, hepatic, pancreatic, cervical, and renal cell lines. Genistein (100 µg/mL; 370 µM) caused dose- and time-dependent inhibition of (SMRT R-1, R-2, R-3 and R-4) renal carcinoma cell lines [36]. Highly metastatic bladder cancer cells (253J B–V) treated with a range of various isoflavone concentrations (10–50 µM) for 48 hours showed growth inhibition effects, with genistein being the most effective even at the lowest dose of 10 µM [33].

Role of Phytoestrogens in Inflammation

Inflammation is a biological response of vascular tissue and is important for immune response and wound healing [37]. However, in cancer, inflammation can create a microenvironment around the tumor, resulting in the attraction of chemokines, cytokines, selectins, and tumor-associated macrophages. Instead of their normal function of phagocytosis of tumor cells, they promote cancer progression, matrix degradation, invasion and metastasis [38, 39]. The use of anti-inflammatory drugs has been shown to significantly reduce the colorectal reoccurrence in patients with previous history of colorectal cancer [40]. The role of nonsteroidal anti-inflammatory drugs (NSAID) is well documented in lowering cancer incidence [41–43]. The mechanism of action of NSAIDs on inflammation is through the inactivation of cyclooxygenase-2 (COX-2), a key enzyme for the prostaglandin production

Table 1 Effects of phytoestrogen treatment on the cell cycle.

Nature of isoflavone	Concentration	Cell type	Biological change	Reference
Genistein, glycitein, and daidzein	0, 10, 25, and 50 µM each	human bladder cancer cell line 253JB-V	a) genistein, compared to other isoflavones, significantly inhibited the cell growth b) cell cycle arrest at G2/M transition	Singh et al. 2006 [33]
Genistein	2.5 to 40 µM	cervical cancer cell lines CaSki and ME180	a) dose-dependent inhibition of both cell lines b) cell cycle arrest at G2/M transition in ME180 cell line only	Yashar et al. 2005 [34]
Genistein alone or combined with radiation	15 µM genistein alone or 3 Gy radiation alone or 15 µM genistein + 3 Gy radiation	prostate cancer cell line PC-3 human breast cancer cell line BR231 human renal cancer cell lines KCI-18 and RC-2	genistein combined with radiation compared to either treatment alone a) significantly inhibited cell growth in all cell lines b) significantly greater G2/M cell cycle arrest c) downregulation of cyclin B1 d) upregulation of P21^{WAF1} pretreatment of cells with 30 µM genistein followed by 3 Gy radiation significantly decreased NF-kB DNA binding	Raffoul et al. 2006 [35]
Genistein	0 to 370 µM	human renal carcinoma cell lines SMKT R-1, R-2, R-3, and R-4	dose-dependent inhibition of growth and the 100 µg/L (370 µM) dose resulted in a time-dependent inhibition in all four cell lines	Sasamura et al. 2002 [36]

that plays an important role in inflammation, but the risk of gastrointestinal bleeding remains the major side effect of such treatment. In a recent study, red clover extract showed an anti-inflammatory effect comparable to that of hydrocortisone treatment in an *in vivo* chicken egg chorioallantoic membrane assay (CAM) [44].

Topical treatment with genistein alone or with capsaicin (a bioactive compound from peppers) in Sprague-Dawley rats, pretreated with 100 µM 12-*O*-tetradecanoylphorbol 13-acetate (TPA) to induce an inflammatory response, effectively inhibited COX-2 and all three mitogen-activated protein kinases – pJNK, pERK, and pp38 [44]. Similar effects of genistein alone or in combination with capsaicin were observed in MCF-7 breast cancer cells [45]. In another study C57BL/6 mice were fed either a soy-free control diet or 3 g/kg soy diet (total aglycone content of 298 mg/g in the diet) for 4 weeks, and were then injected with either 0.2 µg/kg body weight lipopolysaccharide (endotoxin) or vehicle. The soy-fed group showed inhibition of the endotoxin by maintaining the glutathione levels [46]. The expression of inflammation-induced genes, metallothionein and manganese superoxide dismutase, were significantly lower in the soy-fed group (**Table 2**). The study revealed the dose-dependent inhibitory effects of genistein on IL-6 secretion in human intestinal Caco-2 cells. In a recent study of a rat model of inflammatory bowel disease, trinitrobenzene sulfonic acid-induced colitis in Wistar rats, a 100 mg/kg body weight oral dose of genistein significantly inhibited myeloperoxidase and COX-2 [47]. More recently, a case control study of gastric cancer patients revealed that a genetic polymorphism of the anti-inflammatory IL-10 gene and low soy intake were associated with an increased risk of gastric cancer compared to no polymorphism and high soy intake [48].

In the rat models of inflammation, soy and soy isoflavone treatment have shown promising results of COX-2 inhibition, without any side effects. In a 16-week soymilk supplementation study among US postmenopausal women, there was a significant reduction in inflammatory markers [49]. Randomized placebo-controlled trials are needed to further evaluate the anti-inflammatory effects of various phytoestrogens.

Role of Phytoestrogens in Angiogenesis

Angiogenesis is the process by which new blood vessels are formed from the existing vasculature. It is essential for normal physiological processes such as growth and wound healing, and is crucial in tumor progression [50, 51]. Vascular endothelial growth factor (VEGF) is a potent stimulator of angiogenesis. Anti-angiogenic drugs aim to inhibit tumor vascularization. The soy isoflavones genistein, daidzein, orobol (3′-hydroxygenistein) and factor 2 (6,7,4′-trihydroxyisoflavone) (**Fig. 1**) significantly inhibit angiogenesis in the CAM assay [52]. Densitometric evaluation of the blood vessel surfaces showed that, compared to negative controls, genistein and daidzein reduced angiogenesis by 75 % and 48 %, respectively. A recent study demonstrated that the soy isoflavones daidzein and genistein, as well as red clover extract, exerted antiangiogenic effects comparable to that of thalidomide (a potent antiangiogenic) in the CAM assay [44]. Although the CAM assay and the corneal assay are highly sensi-

Table 2 Effects of phytoestrogen treatment in animal models of cancer.

Nature of isoflavone	Amount	Animal model	Biological response	Reference
Genistein or genistein with capsaicin	topical treatment with 25 µM genistein or capsaicin alone or combined in the rats, and 50 µM each alone or together in the cell culture	Sprague-Dawley rats treated with 100 nM TPA MCF-7 breast cancer cells treated with 20 nM TPA	inhibition of COX-2, pJNK, pERK, and pp38	Hwang et al. 2009 [45]
Novasoy diet	diet supplemented with 0.3 % Novasoy	male C57BL/6 mice fed the experimental diet followed with 0.2 µg/kg body weight endotoxin injection	Novasoy treatment resulted in inhibition of the endotoxin.	Paradkar et al. 2004 [46]
Genistein	100 mg (370 µmol)/kg body weight administered orally	TNBS-induced chronic colitis in Wistar rats	significant reduction in COX-2 and myeloperoxidase	Seibel et al. 2009 [47]
Genistein	human renal carcinoma cells (RCC) pretreated with 100 µg/mL (370 µM) genistein for 12 h or genistein 100 µg/mL (370 µM) put in Millipore filter chamber with or without genistein pretreated RCC	C57BL/6 mice with human renal carcinoma cells injected in dorsal air sac	No effect of genistein pretreatment on vascular volume. Genistein in Millipore without genistein pretreated RCC decreased vascular volume by 48.9 %. Genistein in Millipore with genistein pretreated RCC decreased vascular volume by 56.4 %.	Sasamura et al. 2004 [53]
Genistein or soybean-based diet	*in vitro* 1–100 µM genistein	*in vitro* B16F0 melanoma F3II mammary carcinoma cell lines	no cytotoxicity with 100 µM in both cell lines	Farina et al. 2006 [54]
	in vivo 15 mg (55.5 µmol)/kg body weight genistein injection or soybean-based diet	*in vivo* Balb/c and C57BL/6 mice injected with B16 or F3II cells	1–50 µM, and 20–50 µM genistein significantly reduced tumor cell migration in F3II and B16F0 cells, respectively. Both treatments reduced angiogenesis in mice.	
Isoflavone mixture or genistein followed with 5 Gy photon radiation	50 mg/kg body weight isoflavone mixture 21.5 mg (80 µmol)/kg body weight genistein	male nu/nu mice with established prostate tumors with PC-3 cells implants	Isoflavone mixture with radiation inhibited 86 % of prostate tumor growth. This inhibition was similar to that of genistein followed by radiation.	Raffoul et al. 2007 [61]

tive assays to detect new vasculature, there is a large difference between these assays and an actual tumor environment. To address this issue, Sasamura et al. conducted a study with renal cell carcinoma cells injected in the female C57BL6 mice dorsal air sac, and treated them *in situ* with 100 µg/mL (370 µM) genistein which caused a 50% reduction in new vasculature compared with the vehicle [53]. As noted by the authors, this interesting result would have greater importance if genistein was antiangiogenic when using much lower concentrations (1–10 µM), consistent with therapeutic doses.

Hormone-independent mammary tumor cells (F3II) and melanoma cells (B16F0) treated with a very high concentration (100 µM) of genistein showed no cytotoxicity, and a lower concentration (1–50 µM in F3II cells and 20–50 µM in B16F0 cells) showed a significant reduction in tumor cell migration. The tumor cells were injected intradermally in C57BL/6 and BALB/c mice, which were treated either with a soy-based diet or a daily dose of 15 mg (55.6 µmol)/kg body weight genistein. Both treatments significantly reduced the new vasculature as compared to the control group [54]. In human breast cancer xenografted BALB/c nu/nu mice, treatment with a phytoestrogen supplemented diet as 10% ground flaxseed, or injected with the lignans enterodiol and enterolactone with 15 mg (50 µmol)/kg body weight showed a significant decrease in tumor growth, extracellular VEGF, and tumor vasculature as compared with controls [55].

Phytoestrogens exert antiangiogenic effects through the inhibition of VEGF. Since the antiangiogenic effect of genistein is stronger as compared to other isoflavones, it is not known whether or not other phytoestrogens will have the similar effects on angiogenesis and if their mode of action will be through VEGF or some other mechanism.

Role of Phytoestrogens in Reducing Metastases

Metastasis is a multistep process by which tumor cells detach from the growing tumor mass, and migrate to form secondary tumors in distant tissues and organs of the body [56]. A large proportion of cancer mortality is due to deaths from metastases rather than from primary tumors. Phytoestrogens have shown promising results in this area of cancer therapy. Hydroxymatairesinol (HMR), a plant lignan and its metabolite enterolactone (ELN), a mammalian lignan, inhibit cell proliferation and invasion in rat hepatoma cells (AH109A) at ≥ 50 µM and > 200 µM, respectively. A similar significant inhibitory effect was observed with serum from rats fed HMR [15 mg (40 µmol)/100 g body weight] in an AH109A cell culture [57]. Many studies have reported the benefit of phytoestrogens to enhance the efficacy of cancer chemotherapeutics. A 10-week treatment with either soy phytochemical concentrate or genistin (the *β*-glucoside form of genistein present in soy) in SCID mice xenografted with human bladder tumor cells showed a significant 52% and 54% tumor reduction, respectively. Both treatments significantly reduced lung metastasis; however, no effect was observed for lymph node metastasis [33]. BALB/c mice transplanted with undifferentiated human pancreatic carcinoma cells with great metastatic potential (MIA PaCa-2) and treated with a daily 1.3 mg intraperitoneal dose of genistein significantly inhibited distant metastasis, and improved survival as compared to the controls [58]. In a bone metastatic prostate cancer mouse (SCID) model, where mice were injected with the human PC3 cancer cells, a combined treatment of genistein with the chemotherapeutic docetaxel showed significant inhibition of tumor growth, downregulation of MMP9, and significant inhibition in invasion, compared to monotherapy with either compound alone [59]. In a postsurgical orthotopic breast cancer model where mammary fat pads of nude mice were injected with human ductal carcinoma cells (MDA-MD-435/HAL) with high lung metastatic potential, genistein treatment [750 µg/g of diet – an approximate daily dose of 45 mg (180 µmol)/kg body weight] for 70 days resulted in a significant inhibition in lung metastasis. Adjuvant treatment with genistein along with primary tumor resection using the above cancer model revealed a 100-fold reduction in metastatic burden [60]. In both experiments genistein treatment significantly reduced cell proliferation and increased apoptosis within the lung metastasis.

Two studies where orthotopic prostate cancer bearing mice showed spontaneous metastasis to the lymph nodes upon treatment with genistein [oral dose of 5 mg (18.5 µmol)/day] alone as compared to genistein combined with radiation (5 Gy photons as single dose) have raised the question about its safety [61, 62]. However, the median survival was similar in control and genistein-treated mice, and genistein treatment had no detrimental effects on survival. Nonetheless, genistein combined with radiation exerted a significant increase in survival which was greater than that of radiation alone. Genistein treatment was initiated 13 and 14 days post-tumor cell implantation, and there may be little or no effect when metastasis may have already started. Moreover, the sample size was fairly small (6–7 mice), and the lymph node weight was reported only in the short treatment group, with no data reported from the long-term genistein treatment group [62]. The investigators repeated the experiment and treated the same tumor model with 1 mg/day soy isoflavone mixture (genistein, daidzein, and glycitein) or a lower dose of pure genistein 0.43 mg (1.6 µmol)/day, after the tumor xenograft was established. The treatment with a soy isoflavone mixture alone had no stimulatory effect on the lymph node weight, and there was no significant effect for genistein alone. Genistein or a soy isoflavone mixture as a combined therapy with radiation significantly inhibited metastasis [61]. Similarly, in a study of a human prostate cancer mouse BALB/c model, treatment with either 100 mg (370 µmol) or 250 mg (926 µmol)/kg of diet of genistein started prior to the cancer xenograft showed a significant reduction in lung metastasis, and no difference was observed for lymph node weight compared to control [63]. HMR treatment (0.15% of diet) for 14 days in Donryu rats (4 weeks of age) bearing hepatoma resulted in significantly lower tumor weights and smaller tumors, and the rats had no metastasis. Similar effects of ELN treatment (0.001% and 0.01%) were observed on tumor weights and metastasis [57].

There are several ongoing phase I, II, and III clinical trials on phytoestrogens as adjuvant therapy in cancer patients undergoing surgery, radiation, or hormonal manipulations for evaluation of the safety, and effectiveness (**Table 3**) [64].

In summary, phytoestrogens in animal models of breast, prostate, bladder, and pancreatic cancer have shown significant inhibition of distant metastases. Phytoestrogen therapy along with primary treatment either with docetaxel or tumor resection showed significant improvement in metastasis inhibition. The animal data are encouraging, and phase I and II clinical trials are ongoing in this area.

Phytoestrogens Enhance the Efficacy of Cancer Therapy

Overcoming drug resistance is one of the challenges for anticancer therapies, as with time cancers develop resistance to chemotherapeutics, which is in part due to the activation of nuclear factor kappa B (NFκB). Many antitumor drugs act as stimulators for NFκB activation and DNA binding that is responsible for the drug resistance [65]. NFκB plays an important role in cell survival by protecting cells from apoptosis. Prostate cancer cells (PC-3) treated with a low-dose 3 Gy photon radiation exhibited significantly increased NFκB-DNA binding. However, pretreatment with a 30 µM genistein dose for 24 hours prior to the irradiation significantly reduced the NFκB-DNA binding by 66% [35]. Genistein treatment in a number of cancer cell lines including prostate, breast, renal cell carcinoma, as an adjuvant therapy followed by radiation, showed a greater effect than either therapy alone. Pretreatment with 15–30 µM genistein in prostate, breast, lung, and pancreatic cancer cell lines significantly inhibits NFκB DNA binding and enhances the action of antitumor drugs by greater inhibition of cell proliferation and induction of apoptosis [66]. Genistein regulates the Akt signaling pathway [66]. Akt plays a critical role in cell survival and apoptosis, and regulates the NFκB pathway [67], suggesting dual role of genistein on NFκB.

In a metastatic prostate cancer model, PC-3 cancer cells implanted in the prostate of male nude mice, isoflavone therapy (50 mg/kg body weight/day) or an equivalent genistein dose [(0.43 mg (1.6 µmol)/day)] along with radiation significantly inhibited prostate tumor growth compared with control, or either therapy alone. Although isoflavone treatment alone significantly inhibited the tumor growth [61], another study utilized the prostate cancer orthotopic model and demonstrated that a 4-week treatment with 5 mg/day genistein given in combination with 5 Gy photon radiation resulted in a greater magnitude of tumor inhibition, and a significant increase in survival than either therapy administered alone [62]. In contrast, a high 5 mg (18.5 µmol)/day dose of genistein to treat orthotopic renal cell carcinoma in nude mice resulted in a nonsignificant increase in tumor weight, and showed no effect to radiation at a dose of 8 Gy. However, when radiation and genistein were given together, there was a significant inhibition of kidney tumor growth compared to control or either therapy alone [68]. Nonetheless, the Shanghai Breast Cancer Study reported an adverse outcome with radiotherapy compared to no radiotherapy in a population known to consume high amounts of soy food. However, the results may be skewed by the fact that radiation therapy was given to the most advanced staged breast cancer patients and no data were reported on dose and frequency of radiation [69].

In summary, phytoestrogens play an important role in NFκB regulation. Animal studies support the benefit of a combined regimen in cancer therapy as compared to radiation alone on tumor growth. Randomized clinical studies are needed to evaluate the extent of their chemotherapeutic potential in cancer patients.

Randomized Clinical Trials of Phytoestrogens in Cancer Patients

High phytoestrogen intake is well documented among Asian women, and the epidemiological evidence for the long-term intake of such compounds has shown them to be both safe and protective against many diseases including cancer. There has been an open question whether or not phytoestrogens are safe to be used among cancer patients [70, 71]. Little is known about phytoestrogen intake among cancer patients. A recent cross-sectional study of 100 women treated for breast cancer at the Cancer Treatment Centers of America reported that 65% of patients have had some soy food with the mean daily intake of 11.6 mg (43 µmol) genistein and 7.4 mg (29 µmol) daidzein. The isoflavone dose is equivalent to a daily intake of ¼ cup of tofu, and is higher than the usual intake of isoflavones among non-Asian women [72], and the

Table 3 Ongoing clinical trials of phytoestrogens in cancer patients.

Currently ongoing Study	Phase	Condition	Intervention	ClinicalTrials.gov identifier
Flaxseed and/or anastrozole in treating postmenopausal women undergoing surgery for newly diagnosed stage I or II breast cancer	pilot study	newly diagnosed primary, invasive breast cancer estrogen receptor-positive tumor	flaxseed, anastrozole, or placebo	NCT00635908
Genistein in treating patients undergoing external-beam radiation therapy for bone metastases	phase I; phase II	histology confirmed malignant solid tumor, including any of the following: breast cancer, kidney cancer, lung cancer, prostate cancer, or melanoma	genistein, radiation therapy, and quality of life assessment	NCT00769990
Vitamin D and soy supplements in treating patients with recurrent prostate cancer	phase II	histology confirmed adenocarcinoma of the prostate	cholecalciferol genistein, and soy isoflavones	NCT00499408
Genistein in treating patients with pancreatic cancer that can be removed by surgery	phase II	pancreatic adenocarcinoma	genistein, and therapeutic conventional surgery	NCT00882765
Genistein in patients undergoing surgery for bladder cancer	phase II	bladder cancer	genistein versus placebo	NCT00118040
Clinical trial of purified isoflavones in prostate cancer: comparing safety and effectiveness	phase II	localized prostate cancer	purified isoflavones	NCT01036321
Randomized study of soy protein/isoflavones and venlafaxine on vasomotor symptoms in patints with prostate cancer undergoing hormonal manipulations	phase III	histology confirmed prostate cancer	soy protein/isoflavone powder, venlafaxine, or placebo	NCT00354432

lack of evidence on potential harm suggests that moderation may be the key to their use [73].
A study where 20 prostate cancer patients were treated with purified genistein [300 mg (1.11 mmol)/day] for 28 days and with 600 mg (2.22 mmol)/day thereafter for another 56 days, showed no genotoxic effects, no changes in the micronuclei number and no chromosomal damage were observed when compared with pre-treatment status [74]. Recently, a randomized, double-blind, placebo study demonstrated no effect of a 12-week treatment with 20 g soy protein powder containing a high dose of phytoestrogens (160 mg total soy isoflavones) on cognition, sleep quality, sexual function and quality of life, in prostate cancer patients undergoing androgen deprivation therapy [75]. The investigators reported that soy protein or milk powder (placebo) was mixed in beverages. However, no data were reported on dietary intake; it is not known whether or not the subjects in both arms had any soy isoflavone intake through diet or supplementation prior to or during the study. The small sample size (16 subjects in the placebo, and 17 in the intervention arm) and absence of any measures of isoflavone and their metabolite levels in body fluids (urine or blood) makes it difficult to interpret the results. However, the high dose was well tolerated and there were no reports of any adverse effects from the intervention among prostate cancer patients. Similarly, no detrimental effects of soy powder treatment were observed in a pilot study with 18 prostate cancer patients receiving a two-step intervention to reduce fat intake to less than 15% of daily calories, followed by soy powder supplementation. In the median follow-up time of 10.5 (range 3–45) months, only 2 subjects successfully attained the fat calorie reduction of the study goals; also, the length of the soy supplementation was not clear, whether or not it was carried throughout the follow-up time. There was no information on the wide follow-up range of 3 months to 45 months, and no information on how many subjects were followed-up for short- or long-term periods [76]. In another study, 100 patients with isolated high-grade prostatic intraepithelial neoplasia were supplemented with a 100 mg soy isoflavones along with 200 µg selenium and 60 mg vitamin E for 6 months. Two subgroups in the study population were identified; the larger group (68% of subjects) with a significant decrease in prostate specific antigen (PSA) levels from baseline, and only 16% men with diagnosed prostate cancer. A small proportion of subjects had elevated PSA levels after the intervention, and 25% of the men in this group were diagnosed with the cancer. However, in the absence of a control group, it is not clear whether the PSA levels changed due to the phytoestrogens or the underlying disease progression over time [77]. A phase II, randomized, placebo-controlled trial of 80 mg/day isoflavone treatment among localized prostate cancer patients for 12 weeks did not find any significant effects on serum hormone binding globulin, total estradiol, or testosterone [78, 79]. Nonetheless, the isoflavone dose resulted in a significant increase in serum isoflavone levels among the intervention arm without any cytotoxicity or harmful effects [78, 79].
A randomized, placebo-controlled, double-blind study of flaxseed supplementation for a month in postmenopausal breast cancer patients showed a significant reduction in tumor cell proliferation, c-erb B2 expression, and increased apoptosis [80]. The study used whole wheat flour for the placebo group, which is known to have a high lignan content [23, 24]. The effect size may have been stronger if the placebo were a true placebo (no lignans). In a crossover design 7 postmenopausal breast cancer survivors were given supplementation with 138 mg soy isoflavones per day for 24 days, and following a two-week washout period, the subjects were crossed over to the placebo group. The subjects were blinded to the intervention and the order of the treatment was random. Changes in the superoxide dismutase (SOD1) enzyme were measured as a surrogate marker of antioxidant activity of isoflavones. The treatment significantly increased erythrocyte SOD1, and the increased levels were associated with a lower risk for breast cancer recurrence [81] (**Table 4**).

Role of Phytoestrogens on Survival

In transgenic mice with prostatic adenocarcinoma, genistein treatment at a dose of 250 (0.93 mmol) or 500 mg (1.85 mmol)/kg diet for 28 weeks inhibited poorly differentiated cancer, improved survival, and significantly reduced osteopontin (OPN, extracellular matrix protein secreted by the macrophages) compared to the mice fed a genistein-free diet [82, 83]. Similarly, genistein given as a preventive treatment (in mice 4–20 weeks of age) prior to tumor initiation reduced OPN dose-dependently, but when genistein treatment was started on 12-week-old mice, a lower concentration increased OPN levels [84]. The elevated level of OPN is associated with tumor progression and increased metastatic potential. A recent study of targeting OPN by anti-OPN antibody in nude mice bearing highly metastatic breast cancer demonstrated the increased tumor latency and inhibited lung metastases [85]. There is an inverse relation between OPN levels and survival among prostate cancer patients [86].
Only a little is known about the effect of phytoestrogens on cancer survival in patients. We were able to locate three studies of such a nature. The first study was conducted among women from Long Island, California, USA; all-cause mortality significantly decreased for subjects in the highest vs. lowest quintile of isoflavone intake among both postmenopausal and premenopausal women. There was a modest reduction in breast cancer mortality [87]. The other was the follow-up study from the Shanghai breast cancer cohort study where the total soy food intake was analyzed as soy protein or isoflavones, and showed no association with breast cancer survival. However, there were no data on the type of chemotherapeutics used, and the distribution of tamoxifen use among the study population [69]. The null association at least assures no harm associated with the use of soy in breast cancer patients. However, more recently, the Shanghai breast survival study, a large population-based follow-up cohort of breast cancer survivors in China, showed that soy protein or isoflavone intake was significantly associated with decreases in cancer recurrence and mortality. The inverse association was observed regardless of estrogen receptor or tamoxifen users and menopausal status, suggesting their safe use among breast cancer patients [88]. This result is tempered by previous findings that adolescent exposure to soy (as tofu consumption) is conditional for the lowered adult risk of breast cancer [89, 90]. Therefore, while benefits of soy with respect to lowered recurrence and prolonged survival occurred in these Chinese women, it cannot be assumed that this result will apply to groups who did not have early life exposure to soy and its isoflavones.

Table 4 Clinical trials of phytoestrogens.

Clinical stage of the cancer patients	Sample size	Nature of phytoestrogen treatment	Dosage	Length of treatment	Side effects	Results	Limitations	Reference
Stages B, C, or D Prostate neoplasia	N = 20	purified genistein	a) 300 mg (1.11 mmol)/d followed by b) 600 mg (2.22 mmol)/d	a. 28 days b. 56 days	not reported in the study	no genotoxic effects observed	The goal of the study was to evaluate safety and there were no data reported on efficacy of the treatment.	Miltyk et al. 2003 [74]
Prostate cancer (clinical stage not reported)	N = 33, placebo 16, treatment 17	20 gm revival soy protein whole milk protein as placebo	64 mg (237 µmol) genistein, 63 mg (248 µmol) daidzein, and 34 mg (120 µmol) glycitein 20 gm of protein/d	12 weeks	none	no benefit on cognition, sleep quality, sexual function or quality of life	small sample size, no dietary data, did not measure biomarkers; the length of treatment may not be sufficient to observe any differences	Sharma et al. 2009 [75]
Prostate cancer (no stage reported)	N = 17	soy powder, in addition, Vit E, selenium and multivitamin	70.39 mg (261 µmol) genistein, 36.87 mg (145 µmol) daidzein, and 6.70 mg (24 µmol) glycitein, 400 IU/d vitamin E; 200 µg/d selenium	not specified and 2 months	none	no significant decrease in PSA levels	small sample size, no specified length of soy treatment	Spentzos et al. 2003 [76]
High-grade prostatic intraepithelial neoplasia	N = 100	prevalon tablet; in addition, 100 µg selenium, 30 mg vitamin E	42 mg (156 µmol) genistein, 35.2 mg (139 µmol) daidzein and 22.8 mg (80 µmol) glycitein 200 µg/d selenium, and 60 mg/d vitamin E	6 months	not reported in the study	Two subgroups were identified: one group with decreasing or stable PSA with 25 % risk of finding prostate cancer, second group with increasing PSA and 52.2 % risk of finding prostate cancer.	A high drop-out rate; at the 6-month follow-up the study had only 58 subjects; it remains questionable whether or not PSA is a good marker for prostate cancer risk and in this study if the levels change due to phytoestrogen treatment or simply the disease progression over time.	Joniau et al. 2007 [77]
Localized prostate cancer	N = 53, placebo 28, treatment 25	prevastein HC pills (soy based isoflavone concentrate), in addition, multivitamin	80 mg/day (2 pills). Isoflavone content of the pills not provided	12 weeks	Two subjects dropped out due to grade 1 to 2 adverse events (mild to moderate side effects including GI symptoms such as gas, bloating, loss of appetite, dyspepsia, and diarrhea) grade I event reported by 5 subjects in treatment and 7 in placebo; no clinical toxicity.	no significant effects on serum hormone binding globulin, total estradiol, or testosterone		Kumar et al. 2007 [78, 79]
Primary breast cancer	N = 32, placebo 13, flaxseed 19	flaxseed supplemented muffin	25 g/day flaxseed	13–55 days		significantly reduced cell proliferation, and c-erbB2 expression, increased apoptosis	A wide treatment range; lack of a true placebo group (whole wheat flour was given to the placebo group that is known to have considerable amount of lignans).	Thompson et al. 2005 [80]
Breast cancer survivors	N = 7	soy isoflavone concentrate) and placebo	15 mg (55.5 µmol) genistin + genistein, 81 mg (319 µmol) daidzein + daidzein, and 42 mg (148 µmol) glycitin + glycitein	crossover study – soy isoflavones and placebo for 24 days each with 2 weeks wash-out time between the two	none reported	significant increase in erythrocyte SOD1	short intervention time and small sample size	diSilvestro et al. 2005 [81]

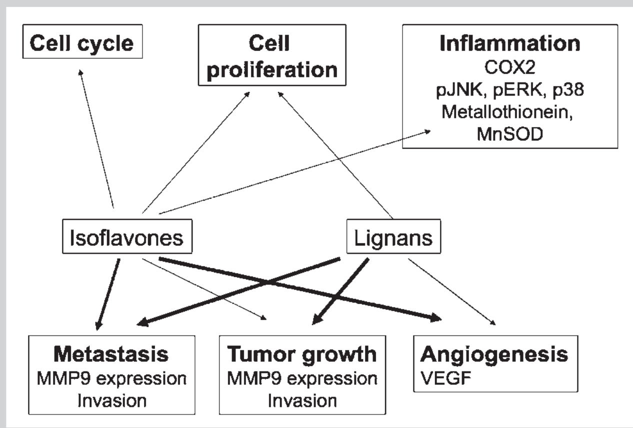

Fig. 3 Summary of the targets of phytoestrogens. The majority of the effects were identified in experiments using the isoflavone genistein. The lignans have qualitatively different effects to the isoflavones. The majority of the effects were negative in nature. However, genistein stimulates the proliferation of estrogen receptor-positive cells at low (< 1 µM) concentrations. The boldness of the arrows indicates the strength of the data as determined by this review.

Conclusions

There is evidence for the role of phytoestrogens in cell cycle arrest, antiangiogenic potential, antimetastatic potential and in enhancing radiotherapy treatment. In the rodent cancer models phytoestrogens significantly inhibit tumor growth, invasion and metastasis (▶ **Fig. 3**). Phytoestrogens have shown to be beneficial in reducing the resistance to anticancer drugs through regulating NFκB. The objective measures of phytoestrogen intake compliance are lacking in most of the clinical trials and wide ranges (80–600 mg/day) of doses have been used among cancer patients. However, the lack of side effects at the highest dose [600 mg (2.22 mmol)/day] of pure genistein at least assures their safe use among this population. More recently, high phytoestrogen intake has been shown to reduce cancer recurrence and mortality among breast cancer survivors. However, the minimum effective dose and whether it is going to differ according to the stage and type of cancer are yet to be determined. The individual differences in metabolism may play another important role in determining the effective dose. It is not known whether these effects of phytoestrogens would vary by obesity status and degree of obeseness. The use of phytoestrogens in cancer therapy holds potential for further research.

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