Published in final edited form as:
Cancer Lett. 2008 October 8; 269(2): 226–242. doi:10.1016/j.canlet.2008.03.052.

MULTI-TARGETED THERAPY OF CANCER BY GENISTEIN

SANJEEV BANERJEE, **YIWEI LI**, **ZHIWEI WANG**, and **FAZLUL H. SARKAR***
Department of Pathology, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201

Abstract

Soy isoflavones have been identified as dietary components having an important role in reducing the incidence of breast and prostate cancers in Asian countries. Genistein, the predominant isoflavone found in soy products, has been shown to inhibit the carcinogenesis in animal models. There is a growing body of experimental evidence showing that the inhibition of human cancer cell growth by genisteinis mediated via the modulation of genes that are related to the control of cell cycle and apoptosis. It has been shown that genistein inhibits the activation of NF-κB and Akt signaling pathways, both of which are known to maintain a homeostatic balance between cell survival and apoptosis. Moreover, genistein antagonizes estrogen- and androgen-mediated signaling pathways in the processes of carcinogenesis. Furthermore, genistein has been found to have antioxidant properties, and shown to be a potent inhibitor of angiogenesis and metastasis. Taken together, both *in vivo* and *in vitro* studies have clearly shown that genistein, one of the major soy isoflavones, is a promising agent for cancer chemoprevention and further suggest that it could be an adjunct to cancer therapy by virtue of its effects on reversing radioresistance and chemoresistance. In this review, we attempt to provide evidence for these preventive and therapeutic effects of genistein in a succinct manner highlighting comprehensive state-of-the-art knowledge regarding its multi-targeted biological and molecular effects in cancer cells.

Keywords

Genistein; Akt; NF-κB; chemoprevention; chemosensitization; cancer therapy

1. Introduction

Laboratory research backed by epidemiological studies emancipating from the last few decades have provided convincing evidence that isoflavones in soy rich foods contribute to relatively lower rates of prostate and breast cancers in Asian countries such as China and Japan than in Western population. Genistein (4,5,7-trihydroxyisoflavone) has been identified as the predominant isoflavone in soybean enriched foods which comprises a significant portion of the Asian diet, and provides 10% of the total per capita protein intake in Japan and China. A recent study among women in Shanghai, China found that plasma isoflavone concentration were inversely associated with the risk of non-proliferative and proliferative benign fibrocystic conditions as well as breast cancer [1]. In parallel, relatively high levels of soy isoflavones

*Corresponding author: Fazlul H. Sarkar, Ph.D., Department of Pathology, Karmanos Cancer Institute, Wayne State University School of Medicine, 740 Hudson Webber Cancer Research Center, 110 E Warren, Detroit, MI 48201, Phone: 313-576-8327; Fax: 313-576-8389; E-mail: fsarkar@med.wayne.edu.

have been found in the serum, urine and prostatic fluid of Asian men who consume a soy rich diet possibly contributing in lowering the incidence of prostate cancer [2;3]. Since these initial findings, several subsequent reports have been published documenting decreased risk of localized prostate cancer associated with soy product and isoflavone consumption [4]. High consumption of soymilk has also been associated with reduced risk of cancer [5]. These facts provide an excellent opportunity for primary prevention of the most common cancers worldwide. Furthermore, isoflavone sensitizes the effect of radiotherapy and cytotoxic chemotherapeutic drugs used in variety of cancers, thus opening avenues for devising novel therapeutic options.

We attempt herein to summarize the known inhibitory effects of genistein on cancer cells and provide a comprehensive review on the multi-targeted molecular mechanism(s) underlying the chemopreventive and therapeutic actions of genistein.

1.1. Molecular structure, estrogenic activity and metabolism of genistein

The isoflavone genistein was originally identified as having a close similarity in structure to estrogens and harboring weak estrogenic activity and, as such, was labeled as a phytoestrogen. The basic structural feature of isoflavone compounds is the flavone nucleus, which is composed of 2 benzene rings linked through a heterocyclic pyrane ring. Because of its structural similarity to 17β-estradiol, genistein has been shown to compete with 17β-estradiol in ER binding assays. Kuiper et al. [6] reported that the binding affinity of genistein for ER-α was 4%, and for ER-β was 87%, compared to estradiol. Thus, by interaction with estrogen receptor, genistein blocks the binding of more potent estrogens at the same time and affects estrogen metabolism, thereby exerting a potential favorable role in the prevention of hormone related cancers.

After intake and ingestion, genistein along with other isoflavones is conjugated with glycoside and metabolized by the enzymes of the intestine. It has been proposed that in humans, genistein is metabolized to dihydrogenistein and 6′-hydroxy-O-desmethylangolensin. Genistein and their metabolites have been detected in plasma, prostatic fluid, breast aspirate and cyst fluid, urine, and feces [2;3;7;8]. Adlercreutz et al. [2] have found that the plasma level of genistein in people having a soy rich diet was 1–5 μM after metabolism and excretion. A recent report from India also revealed an adequate circulating level of genistein after a single dose of soy extract [9]. Another study targeted Phase I pharmacokinetic and pharmacodynamic analysis following administration of unconjugated soy isoflavones (containing 43% and 90% genistein respectively), to individuals with cancer found plasma concentration of genistein supposedly associated with antimetastatic activity *in vitro* [10]. Genistein is relatively hydrophobic and expected to be taken up by cells without previous cleavage and does not have to be biologically active to exert its inhibitory effects on cancer cell growth [11]. However, cancer cell-specific concentration of genistein in human population has not been determined.

1.2. Biological effects of genistein

Many important biological effects of genistein consumption have been elucidated with respect to its anticancer properties. Nevertheless, genistein has many other important health benefits, such as lowering the incidence of cardiovascular diseases [12], prevention of osteoporosis, and attenuation of postmenopausal problems [13]. Furthermore, it has been reported that genistein decreases body mass and fat tissue, accompanied by a decreased apetite. After ingestion of dietary genistein, alterations in concentrations of hormones such as insulin, leptin, thyroid hormones, adrenocorticotropic hormone, cortisol and corticosterone were observed. Additionally, genistein intake is also associated with altered expression of genes engaged in lipid metabolism and disturbed glucose transport into cells affecting lipolysis, lipogenesis and altered ATP synthesis. These metabolic and hormonal changes have been succinctly summarized by Szkudelska and Nogowski in a review article [14].

Genistein is a known inhibitor of protein-tyrosine kinase (PTK), which may attenuate the growth of cancer cells by inhibiting PTK-mediated signaling mechanisms [15]. Sakla et al recently reported that genistein inhibits the protooncogene HER-2 protein tyrosine phosphorylation in breast cancer cells as well as delaying tumor onset in transgenic mice that overexpress the HER-2 gene This data support its potential anticancer role in chemotherapy of breast cancer [16]. However, effects independent of this activity have also been demonstrated [17]. For example, genistein also inhibits topoisomerase I and II [18], 5α-reductase [19] and protein histidine kinase [20], all of which may contribute to the antiproliferative or pro-apoptotic effects of genistein. It has been found that soy isoflavones, including genistein, have antioxidant effects and protects cells against reactive oxygen species by scavenging free radicals, inhibiting the expression of stress response related genes, thereby reducing carcinogenesis [21;22]. Genistein has been shown to inhibit the growth of both estrogen and androgen receptor positive and negative breast and prostate cancer cells *in vitro*, respectively, and showed inhibitory effect on estrogen-stimulated growth of breast cancer cells [23;24]. Furthermore, we have found that genistein is a powerful inhibitor of NF-κB and Akt signaling pathways, both of which are important for cell survival [25]. These effects of genistein are believed to be involved in the induction of apoptotic processes in genistein-treated cells. Collectively, the knowledge regarding the effects of genistein on cancer cells is rapidly growing although it is clear that genistein is a powerful agent whose utilization for the prevention and/or treatment of cancer is likely be forthcoming.

2. Molecular targets and effects of genistein *in vitro*

Extensive experiments have concluded that genistein functions as a promising chemopreventive agent that inhibits carcinogenesis. Additionally, genistein has been shown to inhibit the growth of various cancer cells through the modulation of genes that are intimately related to the regulation of cell cycle and programmed cell death (apoptosis). Table-1 briefly summarizes multiple molecular targets of genistein action. Genistein also intervenes in several cellular transduction signaling pathways inhibiting carcinogenesis and may also be involved in the regulation of gene activity by modulating epigenetic events such as DNA methylation and/or histone acetylation directly or through the estrogen receptor dependent process [26]. Genistein can also up-regulate mRNA expression of the *BRCA1* gene during mammary tumorigenesis, which is frequently inactivated by epigenetic events in breast cancer [27]. Moreover, it has been demonstrated that the angiogenesis and metastasis could also be inhibited by genistein, implying the pleiotropic effects of genistein on the inhibition of carcinogenesis and cancer cell growth.

2.1. Effects on cell cycle regulation

Experiments have shown that genistein inhibits the growth of several cancer cells including leukemia, lymphoma, ovarian, cervical, leiomyoma, melanoma, neuroblastoma, gastric, pancreatic, breast, and prostate cancer cells [23;24;28–31]. The growth inhibition of cancer cells could be due to cell cycle arrest, which ultimately results in cessation of cell proliferation. It has been demonstrated that genistein induces a G2/M cell cycle arrest in breast cancer, gastric adenocarcinoma and melanoma cells [31;32]. We also showed that genistein induces a G2/M cell cycle arrest in PC3 and LNCaP prostate cancer cells; H460 and H322 non small cell lung cancer cells; MDA-MB-231 and MCF-10CA1a breast cancer cells [33;34]. Genistein also causes G2/M arrest in normal MCF10A breast epithelial cells [35]. However, the effect of genistein was more pronounced in malignant cells compared to normal cells. Thus, it is generally accepted that genistein can cause G2/M cell cycle arrest, but a report has shown that genistein could also arrest mouse fibroblast and melanoma cells at G0/G1 phase of the cell cycle [36]. These data suggests that genistein induces either G2/M or G0/G1 cell cycle arrest, depending on cell lines.

Cell cycle progression is known to be tightly regulated by different cyclins, cyclin dependent kinases (CDKs) and cyclin dependent kinase inhibitors (CDKIs) in different phases of the cell cycle. Cancer cells treated with different concentrations of genistein showed a dose-dependent decrease in the expression of cyclin B, which plays important roles in the positive regulation of CDK activity and is necessary for forming cyclin B/CDK complex during the G2/M phase procession. These observations are in concordance with the G2/M cell cycle arrest, suggesting that genistein-induced cell cycle arrest in cancer cells is partially due to the down-regulation of cyclin B [33;34]. The activities of cyclins/CDKs complexes are negatively regulated by several CDK inhibitors (CDKIs) including $p21^{WAF1}$, $p27^{KIP1}$ and $p16^{INK4a}$. We have found a significant dose-dependent up-regulation of $p21^{WAF1}$ expression in genistein treated cancer cells including MDA-MB-231, MDA-MB-435 and MCF-7 breast cancer cells; PC3 and LNCaP prostate cancer cells; H460 and H322 non-small cell lung cancer cells; and HN4 head and neck squamous carcinoma cells [33;34;37–39]. Touny and Banerjee [40] reported the involvement of upstream kinases Myt-1 and Wee-1 in the transcriptional repression of cyclin B1 and the activation of $p21^{WAF1}$ in prostate cancer cells. They found genistein treatment increased Myt-1 levels and decreased Wee-1 phosphorylation, providing better insight into the possible mechanism of genistein-induced G2/M arrest. These findings closely parallel with results on the inhibition of cancer cell growth and cell cycle arrest, suggesting that genistein can inhibit the growth of cancer cells by modulating the expression of genes that are involved in the regulation of cell growth and the cell cycle.

2.2. Effects on the induction of apoptosis

In addition to cell cycle arrest, another specialized event of genistein action involves the induction of programmed cell death known as ‘apoptosis’. This is mediated by a diverse group of protein moieties in cells, namely the Bcl-2 family, along with a concerted cascade of proteolytic activity of a family of asparate-specific cysteinyl proteases, or caspases activation, leading to the digestion of structural proteins, DNA degradation, and ultimately phagocytosis. The Bcl-2 family is the best characterized group of apoptosis mediating factors and can be divided into two main groups according to their functional properties: anti-apoptotic proteins, for example Bcl-x_L and Bcl-2; and pro-apoptotic proteins, such as Bax, Bak, and Bad. The data from our laboratory showed that genistein could induce apoptosis in MDA-MB-231, MDA-MB-435 and MCF-7 breast cancer cells; PC3 and LNCaP prostate cancer cells; H460 and H322 non-small cell lung cancer cells; HN4 head and neck squamous carcinoma cells, and pancreatic cancer cells [33;34;37–39;41;42]. Using multiple assay techniques as hallmark to detect apoptosis, we found genistein induced apoptosis in all cancer cells tested. Flow cytometry revealed that the number of apoptotic cells increased 43–57% with longer genistein treatment. These results are consistent with studies reported by other investigators [43;44], clearly attesting to the fact that genistein induces apoptosis in cancer cells. This was further corroborated by recent findings of Moiseeva *et.al.*, who reported that physiological concentrations of a dietary phytochemical including genistein results in reduced growth and induction of apoptosis of in cancer cells [45].

To explore the molecular mechanism by which genistein induces apoptosis, we studied the effect of genistein on Bcl-2, Bax and caspases in multiple cell lines and found down-regulation of Bcl-2 protein expression, up-regulation of Bax expression, and activation of caspases after treatment with genistein. Other investigators have also reported that soy isoflavone genistein could induce apoptosis in a variety of human cancer cells through caspase-3 activation and down-regulation of Bcl-2, Bcl-xL, and HER-2/neu [46;47]. Furthermore, the p53 and $p21^{WAF1}$ tumor suppressor genes are also known to be involved in apoptotic processes, and we have detected the expression of p53 gene in MDA-MB-231 breast cancer cells, which are ER-negative and harbor mutant p53. Although the treatment of these cells with genistein down-regulated the expression of the dysfunctional p53, the expression of $p21^{WAF1}$ was induced

within 24 h [37]. These results suggest that the induction of $p21^{WAF1}$ and apoptosis by genistein is functionally operated through a p53-independent pathway. A study reported by Kazi *et al*. [48] showed that genistein induced apoptosis by inhibiting proteosome and induction of $p27^{KIP1}$, IκBα, and Bax. A recent study showed that in hepatocellular carcinoma, genistein induced apoptosis by the activation of several endoplasmic reticulum (ER) stress-relevant regulators, which include the transcription factor-GADD153, *m*-calpain, GRP78 and caspase-12 [49]. Taken together, these findings suggest that ER stress, caspase activation, inhibition of proteosome, down-regulation of Bcl-2, Bcl-xL, and HER-2/neu may partly represent the molecular mechanism by which genistein induces apoptosis, and the existing evidence suggests that many of these cascades may also be regulated either directly or indirectly by nuclear factor-κB (NF-κB).

2.3. Effects on inhibiting the activation of NF-κB

NF-κB plays important roles in the control of cell growth, differentiation, apoptosis and stress response. Under non-stimulating conditions, NF-κB is sequestered in the cytoplasm through tight association with the impeding IκB proteins. Following stimulation, IκB protein is phosphorylated and degraded, allowing the NF-κB to translocate to the nucleus, bind to the NF-κB-specific DNA-binding sites or interact with other transcription factors, and thus regulate gene transcription. We have reported that genistein treatment could modulate NF-κB DNA binding activity in prostate, breast, head and neck, and pancreatic cancer cells by electrophoresis mobility shift assay (EMSA) [37;50;51]. In concordance with our findings Natrajan *et al* also found that in human myeloid leukemia cells genistein blocked activation of NF-κB concomitant with degradation of IκBα [52].

We have further investigated whether genistein could block NF-κB induction by known inducers such as H_2O_2 and TNF-α, both of which has been previously shown to induce NF-κB DNA binding activity. After treatment with H_2O_2 or TNF-α, we observed an increase in NF-κB DNA binding activity in prostate cancer cells, which supports the findings reported by Natrajan *et al.* [52]. However, when the cells were treated with 50 μM genistein for 24 h prior to stimulation with the inducing agent, genistein abrogated the induction of NF-κB DNA binding activity elicited by either H_2O_2 or TNF-α [53]. Furthermore, we found that genistein inhibited the phosphorylation of IκB. By immunohistochemistry and confocal microscopic analysis we also found that the treatment of cells with genistein significantly decreased the nuclear staining of the NF-κB. These results indicate that genistein inhibits the translocation of NF-κB to the nucleus, preventing NF-κB from binding to its target DNA and thereby inhibiting the transcription of NF- κB downstream genes. This process ultimately inhibits cell growth and also induces apoptotic cell death. Although controversies are sprouting whether NF-κB could also function as tumor suppressor gene and thus inactivation of NF-κB could be tumor promoting although the exact role of NF-κB certainly merits further investigation.

It has been reported that in the NF-κB signaling pathway, IκBα is phosphorylated by IκB kinase α (IKKα) and IκB kinase β (IKKβ), while IKK is phosphorylated and activated by the upstream molecule, mitogen activated kinase kinase 1 (MEKK1) [54;55]. We have found that genistein treatment did not alter the protein expression of MEKK1; however, genistein treatment inhibited MEKK1 kinase activity when tested by a kinase assay. These results demonstrate that genistein inhibits MEKK1 activity, which may be responsible for the decreased phosphorylation of IκB, thereby, resulting in the inactivation of NF-κB (unpublished data).

Genistein has also been found to potentiate the antitumor activity of chemotherapeutic agents through regulation of NF-κB. It has been reported that some chemotherapeutic agents such as cisplatin, gemcitabine and docetaxel induce the activation of NF-κB in cancer cells and this may be responsible for drug resistance in cancer cells [42;56;57]. By *in vitro* and *in vivo* studies, we have found that pre-treatment with genistein followed by treatment with lower doses of

docetaxel or cisplatin elicited significantly greater inhibition of cell growth and induction of apoptosis compared to either agent alone [41;42;56-58]. By EMSA, we found that NF-κB activity was significantly increased by docetaxel, gemcitabine or cisplatin treatment, and the NF-κB inducing activity of these agents was completely abrogated in cells pre-treated with genistein. These *in vitro* results were also recapitulated in our *in vivo* studies [41;42;59]. Our results clearly suggest that genistein pre-treatment, which inactivates NF-κB activity, together with other cellular effects of genistein, may contribute to increased cell growth inhibition and apoptosis with non-toxic doses of docetaxel, cisplatin, or gemcitabine.

2.4. Effects on regulation of Akt signaling pathway

Akt signaling is another important transduction pathway that plays a critical role in controlling the balance between cell survival and apoptosis [92]. Evidence suggests that Akt also regulates the NF-κB pathway via phosphorylation and activation of molecules in the NF-κB pathway [60;61]. Thus, strategies to block the activity of Akt would ideally lead to the inhibition of proliferation and the induction of apoptosis. By immunoprecipitation, Western blot and kinase assays we found that genistein treatment reduced the level of the phosphorylated Akt protein at Ser473 compared to control cells, resulting in a dose dependent induction of apoptosis after genistein treatment of cells that display constitutively active Akt [41]. Additional studies were carried out to examine the status of Akt in the PC-3 prostate cancer cells treated with genistein followed by EGF stimulation. We found that EGF treatment alone activated Akt kinase as expected, while genistein pre-treatment abrogated the activation of Akt by EGF [25]. This data demonstrates that genistein inhibits the activation of Akt, which may result in the inhibition of survival signals ultimately leading to induction of apoptotic signals.

We have explored the molecular cross talk between Akt and NF-κB signaling pathways by conducting transfection experiments and found that genistein exerts its inhibitory effects on NF-κB pathway through the Akt signaling pathway [25]. Several reports from other investigators also showed similar regulation between Akt and NF-κB pathways [60–62] and these results strongly suggest molecular cross-talk between NF-κB and Akt pathway and that ‘dual’ disruption of these pathways by genistein could be an effective strategy for the inhibition of cancer cells. Stoica *et al*., demonstrated that genistein exerted inhibitory effect on Akt activation induced by estradiol in MCF-7 cells [63;64]. El Touny and Banerjee [65] recently documented that the chemopreventive action of genistein *in vivo* is mediated through the Akt-GSK-3β signaling downstream effectors retarding cancer progression. Collectively, these results demonstrate that genistein exerts its inhibitory effect on NF-κB signaling through Akt pathway. Thus, abrogation of NF-κB and Akt signaling pathway by genistein may be one of the molecular mechanisms by which genistein inhibits cancer cell growth and induces apoptosis.

2.5. Effects on the regulation of androgen-mediated carcinogenesis

Research on androgens showed that androgens are involved in the development and progression of prostate cancer via activating the androgen receptor (AR) [66]. Prostate-specific antigen (PSA) is a clinically important AR-responsive gene which is used to monitor treatment response, prognosis, and progression in patients with prostate cancer [67]. It has been demonstrated that the transcriptional regulation of PSA occurs via AR binding to the AR-responsive element (ARE) in the promoter region of PSA [68]. The expression of PSA is initially regulated by androgen through the regulation of AR, and undergoes a sharp decline after medical castration [69]. The tumor then becomes androgen-independent and PSA expression is constitutively up-regulated through an unknown mechanism, suggesting the importance of PSA in prostate carcinogenesis.

We have previously demonstrated that genistein has different effects on ARE binding and the expression of AR and PSA in androgen-sensitive (LNCaP) and androgen-insensitive (VeCaP) prostate cancer cells. Genistein transcriptionally down-regulated AR, decreased nuclear protein binding to ARE, thereby, inhibiting the transcription and protein expression of PSA in androgen-sensitive LNCaP cells [70]. Genistein treatment also resulted in a dose and time dependent decrease in the secreted PSA in the media collected from LNCaP cells treated with low concentration of genistein (0.1–5μM). In contrast, genistein did not alter AR expression and binding of nuclear AR to the ARE at low concentration in VeCaP cells. However, higher concentrations (10–50 μM) of genistein were able to significantly inhibit PSA secretion in VeCaP cells. Further studies using transient transfection of a PSA promoter construct revealed that genistein can inhibit PSA synthesis in prostate cancer cells through an androgen-dependent or androgen-independent pathway highlighting the fact that genistein inhibits cell proliferation independent of androgen and PSA signaling pathways. These studies strongly support the role of genistein as a chemopreventive/therapeutic agent for prostate cancer, irrespective of androgen responsiveness. Genistein has also been shown to bind directly to the estrogen receptor and modulate its function [71], suggesting the inhibitory effects of genistein on both androgen and estrogen-mediated carcinogenesis. However, further studies are required to fully understand the complex regulation of ER and AR pathways during genistein induced cell growth inhibition and apoptosis.

2.6. Effects on the regulation of MAPK Pathway

MAPK pathway consists of a three tiered kinase core where MAP3K activates a MAP2K that activates a MAPK (ERK, JNK, and p38), resulting in the activation of NF-κB and cell survival [72;73]. It has been reported that activation of the MAPK pathways may cause the induction of phase II detoxifying enzymes, and inhibition of MAPK pathways may inhibit AP-1-mediated gene expression [74].

Genistein has been found to regulate the molecules in the MAPK pathway. Huang *et al*., reported that genistein inhibited TGF-β–mediated p38 MAP kinase activation, matrix metalloproteinase type 2, and cell invasion in human prostate epithelial cells [75]. In other studies, genistein has been found effective in preventing cytokine- induced ERK-1/2 activation and promoted apoptotic cell death [76;77]. Since genistein is a well known inhibitor of tyrosine kinase, it is possible that genistein may inhibit tyrosine kinase upstream of p38 MAPK and subsequently inhibit the phosphorylation of tyrosine on p38 MAPK, leading to the inactivation of MAPK pathway.

2.7. Anti-oxidation effects

Isoflavones, including genistein, are known antioxidants. Genistein has been shown to protect cells against reactive oxygen species (ROS) by scavenging free radicals and reducing the expression of stress-response related genes [21;22]. It has been demonstrated that genistein inhibits tumor-promoter, 12-O-tetradecanoylphorbol-13-acetate- induced hydrogen peroxide production as well as its function in human polymorphonuclear leukocytes, and HL-60 cells [78;79]. Furthermore, as a follow up to genistein action showing antioxidant capacity, its effect on activation of the transcription factors- Nrf1 and Nrf2, which have been implicated in the regulation of genes involved in response to oxidative stress, was investigated [80], These transcription factors are involved in the regulation of γ-GCS and other detoxification proteins. Genistein was found to induce the cytosolic accumulation and nuclear translocation of Nrf1 and Nrf2 which closely paralleled changes in glutathione peroxidase (GPx) mRNA levels and also the activity of GPx [80]. Genistein has also been shown to stimulate antioxidant protein gene expression in Caco-2 cells [81]. It has been reported that oxidative stress activates NF-κB [82] and our data showed that genistein is an antioxidant by virtue of its inhibition of the activation of NF-κB stimulated by oxidant stress [53]. Thus, the ability of genistein in inhibiting

the generation of ROS, resulting in the inhibition of NF-κB activation, make it a strong candidate as an antioxidant and a powerful chemopreventive agent.

2.8. Regulation of other pathways

Cycloxygenase-2 (COX-2) is a critical enzyme catalyzing synthesis of bioactive prostaglandin E_2 (PGE_2) from the substrate arachidonic acid (AA) and is found to be overexpressed in many human tumor tissues. COX-2 is known to increase cell proliferation and VEGF production, induce angiogenesis, and possess anti-apoptotic effects. Genistein and other soy isoflavones have been found to be effective not only in reducing COX-2 expression but also for antagonizing AA for controlling PGE_2 production and invasiveness of the breast cancer MDA-MB231 cells through downregulation of EGFR and HER-2/neu activity and by modulating the level of NF-κB expression. Further transcriptional control studies by Lau and Leung [83] identified activator protein-1 (AP-1)/cyclic AMP response element binding protein (CREB) binding site in the COX-2 promoter which is critical for COX-2 expression. Genistein suppressed AP-1/CREB binding, resulting in reduced COX-2 expression, which could be important in the post-initiation events of breast carcinogenesis. In addition, genistein has shown to be beneficial in combination with 5-Flurouracil (5-FU) in the treatment of colon cancer through the COX pathway [84].

Genistein also inhibits insulin-like growth factor-1 receptor (IGF-1R) signaling, resulting in the inhibition of cell proliferation and induction of apoptosis. Moreover, Raffoul et al. reported that genistein also enhanced prostate cancer radiotherapy through the downregulation of apurinic-apyrimidine endonuclease 1/redox factor-1 expression [85]. Among the STAT family of transcription proteins, constitutive activation of STAT-3 and STAT-5 has been identified as responsible for cell survival and growth by preventing apoptosis through increased expression of antiapoptotic proteins such as Bcl-2 and Bcl-x_L. Genistein has been shown to inhibit phosphorylation of these transcription proteins which, in turn, may inhibit the constitutive and abnormal signaling cascade, promoting survival and growth of tumor cells [76]. Expression profiling of rat mammary epithelial cells by Su *et al*., [86] confirmed the differential expression of Wnt (Wnt5a, Sfrp2) and Notch (Notch2, Hes1) signaling components by soy protein isolate and/or genistein using quantitative real-time PCR. Wnt pathway inhibition by genistein was supported by reduced cyclin D1 immunoreactivity in mammary ductal epithelium in the genistein treated group, despite comparable levels of membrane-localized E-cadherin and beta-catenin.

3. Inhibition of carcinogenesis *in vivo* by genistein

There is growing *in vivo* evidence demonstrating the inhibitory effects of genistein on carcinogenesis. Although Hawrylewicz et al [87] and Ravindranath et al [88] have published data pertaining to animal studies and anticancer potential of soy isoflavone genistein, here we summarize the *in vivo* studies in a comprehensive fashion.

3.1. Inhibition of cancers in animal

It has been reported that genistein has a protective role against carcinogenesis in animals. Prepubertal exposure to soy or genistein reduced mammary carcinogenesis in rats treated with carcinogens, possibly by modulating the development of the mammary end buds [27;89]. One of the early studies revealed that soy-containing diets reduced the severity of prostatitis in rats [90]. Soy isoflavone supplemented diets also prevented the development of adenocarcinomas in the prostate and seminal vesicles in a rat carcinogenesis model [91]. It has also been reported to be effective in chemical carcinogen-induced rat ovarian carcinogenesis [92]. The soy diet reduced growth of transplantable prostate adenocarcinomas and inhibited tumor cell proliferation and angiogenesis of transplantable prostate cancer in immunodeficient mice

[93;94]. A diet rich in soy also inhibited pulmonary metastasis of melanoma cells in C57Bl/6 mice [95]. Genistein inhibited the growth of carcinogen-induced cancers in rats and human leukemia cells transplanted into mice [87;88;96;97]. Singh *et al* [98] evaluated the natural form of genistein, and the isoflavone-rich soy phytochemical concentrate (SPC) on the growth and metastasis of human bladder cancer cells 253J BV-induced tumors in an orthotopic site. Both treatment regimes were effective in reducing tumor weight by more than 50%, accompanied by induction of tumor cell apoptosis and inhibition of tumor angiogenesis *in vivo*. However, SPC treatment was significantly better, which inhibited lung metastases by 95% and reduced circulating insulin-like growth factor-I levels [98]. Furthermore, genistein protects the skin from the effect of long-term psoralen plus ultraviolet A radiation (PUVA) therapy which is associated with an increased risk of squamous cell carcinoma and malignant melanoma [99; 100]. Additionally genistein is also reported to substantially inhibit skin carcinogenesis and cutaneous aging induced by ultraviolet (UV) light in mice [99]. In a murine PTEN (mPTEN) heterozygous (+/−) mutant mouse model for endometrial carcinoma, as well as in estrogen-related endometrial carcinogenesis, genistein exerted an inhibitory effect on PTEN-related tumorigenesis [101]. Genistein also attenuated gastric carcinogenesis promoted by sodium chloride in a rat model of gastric cancer [102]. Thus, a growing body of literature provide strong evidence to support the role of the various soy products containing genistein in the protection against carcinogenesis in animal models.

3.2. Inhibition of NF-κB activation supporting antioxidant effect of isoflavone in humans

We have investigated the effects of isoflavone supplementation on NF-κB activation *in vivo* in human volunteers [53]. Genistein is an antioxidant as indicated earlier, thus, soy isoflavone supplementation is expected to inhibit NF-κB activation and, in turn, may reduce the oxidative damage in human lymphocytes. The lymphocytes from healthy male subjects were harvested from peripheral blood and cultured for 24 h in the absence and presence of genistein. EMSA revealed that genistein treatment inhibited basal levels of NF-κB DNA binding activity by 56% and abrogated TNF-α induced NF-κB activity by 50%. Furthermore, when human volunteers received 50 mg of soy isoflavone supplements (Novasoy™) twice daily for three weeks, TNF-α failed to activate NF-κB activity in lymphocytes harvested from these volunteers, however lymphocytes from these volunteers collected prior to soy isoflavone intervention showed activation of NF-κB DNA-binding activity upon TNF-α treatment *ex vivo* [53]. These results demonstrate that soy isoflavone supplementation has a protective effect against TNF-α induced NF-κB activation in humans *in vivo*. We have also measured the levels of oxidative DNA damage in the blood of the six subjects before and after supplementation with Novasoy™. The results demonstrate that isoflavone supplementation reduced the levels of the 5-OhmdU and decreased oxidative damage in human subjects, which provided strong evidence that soy isoflavone functions as antioxidant. and these effects of genistein could be responsible for its chemopreventive activity [53].

4. Effects on the inhibition of angiogenesis and metastasis

Matrix metalloproteinases (MMPs) are proteolytic enzymes believed to provide cancer cells with their invasive potential by degrading the extracellular matrix. Genistein has been shown to reduce the angiogenic and metastatic potential of cancers [38;103;104]. Additionally, genistein also significantly decreased the incidence of cancer cell invasion into the lymphatic vessels attenuating cancer metastasis. Our laboratory has examined the inhibitory effect of genistein on tumor cell invasion and metastasis of MDA-MB-435 breast cancer cells transfected with *c-erb*B-2, which has been shown to promote secretion of MMPs and subsequent metastasis in experimental models [38]. We found that the expression of *c-erb*B-2, MMP-2, and MMP-9 in MDA-MB-435 cells stably transfected with *c-erb*B-2, was much higher than that in parental MDA-MB-435 cells. However, the high expression of *c-erb*B-2,

MMP-2, and MMP-9 in MDA-MB-435 435 transfectants was significantly down-regulated by genistein treatment. These results suggest that increased *c-erb*B-2 expression in MDA-MB-435 435 transfectants may result in increased secretion of MMPs, and that genistein may inhibit the expression of *c-erb*B-2 and subsequently decrease the secretion of MMPs in breast cancer cells. An interesting finding reported by Owen *et al*, showed that genistein was effective in decreasing the constitutively high levels of MMP-9 within T-lymphocytes harvested from mammary tumor bearing mice [105].

By gene expression profiling of genistein treated PC-3 prostate cancer cells and PC-3 bone tumor, we also found that genistein down-regulated the expression of MMP-9, MMP-2, protease M, uPAR, VEGF, neuropilin, TSP, BPGF, LPA, TGF-β, TSP-1, and PAR-2, and up-regulated the expression of connective tissue growth factor and connective tissue activation peptide [106]. All of these genes are related to angiogenesis and metastasis. These findings were further supported by studies reporting inhibition of MMP-2 activation and reduction of prostate cancer cell invasion by genistein [75;107]. Another oligonucleotide microarrays study has been reported by Lee wherein the gene expression profile by genistein treatment in breast cancer cells was investigated [108]. Accordingly, this author have shown that TFPI-2, ATF3, DNMT1, and MTCBP-1, which inhibit invasion and metastasis, were upregulated, and MMP-2, MMP-7, and CXCL12, which promote invasion and metastasis, were downregulated.

However, as a corollary to our *in vitro* study, we have further investigated the effect of dietary genistein on the growth of metastatic prostate cancer cells in a SCID-human experimental model of prostate cancer bone metastasis. Our results demonstrate that genistein inhibited prostate cancer cell growth in the bone environment and down-regulated the transcription and translation of genes critically involved in the control of tumor cell invasion and metastasis *in vitro* and *in vivo*, suggesting the possible therapeutic role of genistein for metastatic prostate cancer [109]. Other investigators have also demonstrated similar results showing that isoflavones inhibited bone metastasis of human breast cancer cells in a nude mouse model and metastasis of androgen-sensitive human prostate tumors in mice [110;111]. Furthermore, we documented that genistein also intervenes in the regulation of the osteoprotegerin/receptor activator of NF-κB (RANK)/RANK ligand/MMP-9 signaling in prostate cancer, suggesting that isoflavone genistein could be a promising non-toxic agent augmenting the therapeutic outcome of metastatic prostate cancer with chemotherapeutic drugs [59]. Since cancer metastases follows a multi-step pathway wherein invasion and cell motility is an early step, genistein at physiological relevant concentrations has been shown to be effective in exerting an inhibitory effect on the migration of prostate cancer cells [112]. Complimenting these report, Craft *et.al*., recently demonstrated that genistein also has the potential to therapeutically compensate endoglin deficiency- a key regulator of cell motility in prostate cancer [113]. Another recent report showed that genistein induced metastatic suppressor kangai-1 (KA11), suggesting that genistein could be used for anti-metastatic therapies [114].

Angiogenesis is the formation of new blood vessels and it is essential for normal reproductive function, development and wound repair processes. However, angiogenesis in solid tumors are important and necessary for promoting the proliferation, invasion and metastasis of cancer cells. It has been found that genistein inhibits vessel endothelial cell proliferation and *in vitro* angiogenesis at half maximal concentration of 5 and 150 μM, respectively, suggesting that genistein is a potent inhibitor of vascularization and cancer cell growth [104]. TGF-β is a known major factor that regulates cell proliferation [115], and TGF-β signaling is an important feature in the up-regulation of angiogenesis [116]. Genistein has been known to inhibit TGF-β signaling, and therefore inhibit angiogenesis [116]. Further evidence in support of soy-based foods as natural dietary inhibitors of tumor angiogenesis was reported in a study by Su *et al.* [117]. The efficacy of soy isoflavones on angiogenesis inhibition *in vivo* was examined by nude mice xenograft and chick chorioallantoic membrane bioassay. Factors analyzed included

angiogenic factors, matrix-degrading enzymes, and angiogenesis inhibitors. Genistein was the most potent inhibitor of angiogenesis *in vitro* and *in vivo* among the isoflavone compounds tested. It may also account for most of the reduced microvessel density observed in xenografts and the suppressed endothelial migration by soy isoflavones. Genistein exhibited a dose-dependent inhibition of expression/excretion of vascular endothelial growth factor$_{165}$, platelet-derived growth factor, tissue factor, urokinase plasminogen activator, and matrix metalloprotease-2 and 9, respectively. On the other hand, there was an up-regulation of angiogenesis inhibitors- plasminogen activator inhibitor-1, endostatin, angiostatin, and thrombospondin-1. In addition, a differential inhibitory effect between immortalized uroepithelial cells and most cancer cell lines was also observed. All these reports suggest that tissue factor, endostatin, and angiostatin are novel molecular targets of genistein.

Recently, Guo et al. [118] documented that genistein significantly reduced nuclear accumulation of hypoxia-inducible factor-1α in PC-3 cells, which is the principle transcription factor that regulates VEGF expression in response to hypoxia. These observations support the hypothesis that genistein may inhibit prostate tumor angiogenesis through the suppression of VEGF-mediated autocrine and paracrine signaling pathways between tumor cells and vascular endothelial cells. Hence, we believe that there is ample evidence to suggest that genistein is a potent anti-angiogenic agent and its application in human awaits further investigation.

5. The sensitizing effect of genistein in cancer treatment

In recent years, novel combination treatments with conventional cancer therapies and chemopreventive agents have received much attention in cancer research. More importantly, the published studies have shown that isoflavone genistein could potentiate the antitumor effects of chemotherapeutic agents in various cancers *in vitro* and *in vivo* in preclinical studies. We have reported *in vitro* that genistein potentiated growth inhibition and apoptotic cell death caused by cisplatin, erlotinib, docetaxel, doxorubicin, gemcitabine, and CHOP (cyclophosphamidine, doxorubicin, vincristine, prednisone) in cancers of prostate, breast, pancreas, and lung and lymphoma [56–58;119–121]. We have also found that dietary genistein *in vivo* could enhance the antitumor activities of gemcitabine and docetaxel in a tumor model, resulting in apoptotic cell death and the inhibition of tumor growth [42;109]. Similar observations has been reported by other investigators showing that the antitumor effects of chemotherapeutics, including 5-fluorouracil (5-FU), adriamycin, cytosine arabinoside, tamoxifen and perifosine could be potentiated by genistein [84;122–126]. Genistein also enhanced the antitumor effect of bleomycin in HL-60 cells, but not in normal lymphocytes in an *in vitro* study [127]. The synergistic action of genistein and cisplatin or carmustine (BCNU) on the growth inhibition of glioblastoma and medulloblastoma cells has also been observed [128]. In ovarian cancer, genistein potentiated the antiproliferative and proapoptotic effect of antibodies directed against the cell adhesion molecule L1-CAM [129]. Furthermore, despite limitations in the cytotoxic effect of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) in gastric and pancreatic adenocarcinoma cell lines, subtoxic concentrations of genistein sensitized these TRAIL-resistant cells to TRAIL/Apo2L-mediated apoptosis [130;131]. In radiotherapy, experimental studies from Dr. Hillman's laboratory have demonstrated that the combination of genistein and radiation exert enhanced inhibitory effects on tumor growth and progression of renal cell carcinoma and prostate tumor in orthotopic models [132;133]. Genistein also enhanced radiosensitivity in human esophageal, and cervical cancer cells, suggesting the beneficial effects of genistein in cancer radio-therapy [134;135]. These reports clearly demonstrate that genistein could be used in cancer treatment to further enhance the antitumor activities of conventional therapeutics.

6. Genistein analogues and related studies

To enhance the antitumor activity of isoflavone, several isoflavone derivatives have been synthesized and used in *in vitro* and *in vivo* experiments and in clinical trials. These compounds have shown a low IC_{50} in the inhibition of cancer cell growth *in vitro*. Moreover, at low concentrations, these compounds were able to enhance the antitumor activity of clinically available chemotherapeutic agents, suggesting their potent effects as therapeutic agents for combination treatment. Phenoxodiol is one such analog of isoflavone genistein and has shown a broad-spectrum, anticancer effect. In an animal study, phenoxodiol inhibited dimethyl-benz (a)anthracene (DMBA)-induced mammary carcinogenesis in female Sprague-Dawley rats, suggesting that phenoxodiol is an effective chemopreventive agent against DMBA-induced carcinogenesis [136]. In experimental studies and clinical trials, phenoxodiol has been used both as a mono-therapy and in combination with standard chemotherapeutics. These studies have shown that in some cancers phenoxodiol appears to be strong enough to work on its own as a monotherapy. However, one of the major benefits of phenoxodiol is its ability to sensitize cancer cells to the antitumor effects of conventional chemotherapeutics [137]. It has been found that cancer cells that have become resistant to the effects of conventional chemo-therapeutics, phenoxodiol could restore chemosensitivity [138]. Therefore, by exposing chemoresistant cancer cells to phenoxodiol first, long-standing drug resistance is removed, making cancer cells susceptible once again to standard chemotherapeutics, such as cisplatin, carboplatin, taxanes, and gemcitabine. Phenoxodiol is currently undergoing clinical studies in the USA and Australia. So far, phase I/II clinical trials have shown some disease stabilization without severe toxicity [138]. We have also synthesized structurally-modified derivatives of isoflavone based on the structural requirements for optimal anti-cancer effect [139]. We found that these synthetic derivatives of isoflavone exerted higher anti-cancer activity with lower IC_{50}. These derivatives of isoflavone also induced more apoptosis compared to genistein. Other investigators also synthesized a series of genistein derivatives and evaluated either their cytotoxic potential and/or protective efficacy against hydrogen peroxide induced endothelial cell damage [140;141]. Some of these have effect comparable to 5-Flurauracil in potency of their cytotoxicity [141]. These results suggest that genistein and synthetic structurally-modified derivatives of isoflavone may be promising agents for cancer chemoprevention and therapy either alone or in combination with existing chemotherapeutic agents.

7. Clinical trials: effects on patients with prostate cancer

Several phase I and II clinical trials using isoflavone supplementation have been conducted in the patients with prostate cancer. In phase I clinical trials, the safety, pharmacokinetic parameters, and efficacy of orally administered isoflavones have been determined [10;142; 143]. No toxicity has been observed in the subjects. Oral administration of soy isoflavones gives plasma concentrations of genistein up to 16.3 μM that have been associated with anti-metastatic activity *in vitro* [10]. No genotoxicity has been found in subjects treated with a purified soy unconjugated isoflavone mixture [143].

Our *in vitro* data demonstrated the inhibition of prostate cancer cell growth and decreased PSA expression in LNCaP cells by genistein. Hence, we conducted a phase II clinical trial to investigate the modulation of serum PSA levels in patients with prostate cancer by soy isoflavone supplementation. Patients with prostate cancer were eligible to participate if they had rising PSA levels and were previously untreated (Group I), treated with local therapy (Group II), or treated with hormone therapy (Group III), and had either three successive rising PSA levels or a PSA of >10 ng/ml at two successive evaluation. No other therapy or supplements were allowed during the study period. Patients received 100 mg Novasoy™ (Archer Daniels Midland Company, Decatur, IL, USA) orally twice daily for a minimum of three months in the absence of progression or toxicity. Novasoy™ contains genistein, daidzein,

and glycitin at a 1.3:1:0.3 ratios. Serum PSA, IGF-1 and IGFBP-3 levels were measured and toxicity was assessed. Serum PSA levels were monitored at baseline and monthly during the study. The results showed that soy isoflavone supplementation inhibited the linear rise in PSA in both androgen sensitive and androgen insensitive patient populations. There were no statistically significant changes in the plasma levels of IGF-1 and IGFBP-3. These data demonstrated that soy isoflavone supplementation decreases the rate of rise in serum PSA levels without any toxicity in prostate cancer patients. The lack of significant side effects of soy isoflavone makes it an ideal agent for patients with advanced disease for further studies. Nevertheless, the results of *in vitro* studies along with numerous *in vivo* studies in different animal models, and our pilot *in vivo* human studies collectively point towards a favorable application of genistein as chemopreventive and/or therapeutic agent for prostate and other cancers.

A phase II randomized, placebo-controlled clinical trial using purified isoflavones in modulating steroid hormones in patients with localized prostate cancer has been reported recently [144]. Although significant increases in plasma isoflavones ($P < 0.001$) was observed with no clinical toxicity, the corresponding modulation of serum SHBG, total estradiol, and testosterone in the isoflavone-treated group compared to men receiving placebo was nonsignificant. Increasing plasma isoflavones failed to produce a corresponding modulation of serum steroid hormone levels in men with localized prostate cancer, suggesting the need to explore other potential mechanisms by which prolonged and consistent purified isoflavone consumption may modulate prostate cancer risk [144].

Recently, the investigators from our institute reported the results from a phase II clinical trial designed to investigate the efficacy of lycopene alone or in combination with soy isoflavones on serum PSA levels in men with prostate cancer [145]. 35 of 37 (95%) evaluable patients in the lycopene group and 22 of 33 (67%) evaluable patients in the lycopene plus soy isoflavone group achieved stable disease, described as stabilization in serum PSA level. The data suggest that lycopene and soy isoflavones have activity in prostate cancer patients with PSA relapse disease and may delay progression of both hormone-refractory and hormone-sensitive prostate cancer [145]. Another human *in vivo* study also showed that a daily diet containing four slices of a bread rich in heat treated soy grits favorably influences the PSA level and the free/total PSA ratio in patients with prostate cancer, suggesting the inhibitory effects of phytoestrogen on PSA level [146]. Although these results are provocating, further clinical trials are needed to fully justify the use of isoflavones for cancer prevention and therapy.

8. Conclusions

In conclusion, genistein ingested through natural food sources exerts its anti-carcinogenic effects, mediated via its pleiotropic molecular mechanism(s) of action on cell cycle, cell apoptotic processes, angiogenesis, invasion, and metastasis. These effects may be primarily due to specific effects of genistein on Akt, NF-κB, MMPs and Bax/Bcl-2 signaling pathways. However, further basic and clinical research in this rapidly growing field of isoflavones should provide lessons for its ultimate application in the cancer field. Such advances will provide critical data that will be supported by definitive clinical trials to prove or disprove whether isoflavone, genistein could fulfill its promise as a chemopreventive and/or therapeutic agent against human cancers with utmost confidence.

Acknowledgements

The authors' work cited in this review was funded by grants from the National Cancer Institute, NIH (5R01CA083695, 5R01CA101870, and 5R01CA108535) awarded to FHS, a sub-contract award to FHS from the University of Texas MD Anderson Cancer Center through a SPORE grant (5P20-CA101936) on pancreatic cancer awarded to James

Abbruzzese, and a grant from the Department of Defense (DOD Prostate Cancer Research Program DAMD17-03-1-0042) awarded to FHS.

References

1. Lampe JW, Nishino Y, Ray RM, Wu C, Li W, Lin MG, et al. Plasma isoflavones and fibrocystic breast conditions and breast cancer among women in shanghai, china. Cancer Epidemiol Biomarkers Prev 2007;16:2579–2586. [PubMed: 18086761]
2. Adlercreutz H, Markkanen H, Watanabe S. Plasma concentrations of phyto-oestrogens in Japanese men. Lancet 1993;342:1209–1210. [PubMed: 7901532]
3. Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer 1989;64:598–604. [PubMed: 2743254]
4. Kurahashi N, Iwasaki M, Sasazuki S, Otani T, Inoue M, Tsugane S. Soy product and isoflavone consumption in relation to prostate cancer in Japanese men. Cancer Epidemiol Biomarkers Prev 2007;16:538–545. [PubMed: 17337648]
5. Jacobsen BK, Knutsen SF, Fraser GE. Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States). Cancer Causes Control 1998;9:553–557. [PubMed: 10189040]
6. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998;139:4252–4263. [PubMed: 9751507]
7. Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. Obstet Gynecol 1996;87:897–904. [PubMed: 8677131]
8. Zava DT, Dollbaum CM, Blen M. Estrogen and progestin bioactivity of foods, herbs, and spices. Proc Soc Exp Biol Med 1998;217:369–378. [PubMed: 9492350]
9. Joshi JV, Vaidya RA, Pandey SN, Agashe S, Chandrasekharan S, Menon SK, et al. Plasma levels of genistein following a single dose of soy extract capsule in Indian women. Indian J Med Res 2007;125:534–541. [PubMed: 17598939]
10. Takimoto CH, Glover K, Huang X, Hayes SA, Gallot L, Quinn M, et al. Phase I pharmacokinetic and pharmacodynamic analysis of unconjugated soy isoflavones administered to individuals with cancer. Cancer Epidemiology, Biomarkers and Prevention 2003;12:1213–1221.
11. Russo A, Cardile V, Lombardo L, Vanella L, Acquaviva R. Genistin inhibits UV light-induced plasmid DNA damage and cell growth in human melanoma cells. J Nutr Biochem 2006;17:103–108. [PubMed: 16111876]
12. Si H, Liu D. Phytochemical genistein in the regulation of vascular function: new insights. Curr Med Chem 2007;14:2581–2589. [PubMed: 17979711]
13. Marini H, Minutoli L, Polito F, Bitto A, Altavilla D, Atteritano M, et al. Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. Ann Intern Med 2007;146:839–847. [PubMed: 17577003]
14. Szkudelska K, Nogowski L. Genistein--a dietary compound inducing hormonal and metabolic changes. J Steroid Biochem Mol Biol 2007;105:37–45. [PubMed: 17588743]
15. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 1987;262:5592–5595. [PubMed: 3106339]
16. Sakla MS, Shenouda NS, Ansell PJ, Macdonald RS, Lubahn DB. Genistein affects HER2 protein concentration, activation, and promoter regulation in BT-474 human breast cancer cells. Endocrine 2007;32:69–78. [PubMed: 17992604]
17. Abler A, Smith JA, Randazzo PA, Rothenberg PL, Jarett L. Genistein differentially inhibits postreceptor effects of insulin in rat adipocytes without inhibiting the insulin receptor kinase. J Biol Chem 1992;267:3946–3951. [PubMed: 1310987]
18. Okura A, Arakawa H, Oka H, Yoshinari T, Monden Y. Effect of genistein on topoisomerase activity and on the growth of [Val 12]Ha-ras-transformed NIH 3T3 cells. Biochem Biophys Res Commun 1988;157:183–189. [PubMed: 2848517]

19. Evans BA, Griffiths K, Morton MS. Inhibition of 5 alpha-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. J Endocrinol 1995;147:295–302. [PubMed: 7490559]
20. Huang J, Nasr M, Kim Y, Matthews HR. Genistein inhibits protein histidine kinase. J Biol Chem 1992;267:15511–15515. [PubMed: 1639791]
21. Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP, Rice-Evans CA. Antioxidant activity of phytoestrogenic isoflavones. Free Radic Res 1997;26:63–70. [PubMed: 9018473]
22. Zhou Y, Lee AS. Mechanism for the suppression of the mammalian stress response by genistein, an anticancer phytoestrogen from soy. J Natl Cancer Inst 1998;90:381–388. [PubMed: 9498488]
23. Peterson G, Barnes S. Genistein inhibits both estrogen and growth factor-stimulated proliferation of human breast cancer cells. Cell Growth Differ 1996;7:1345–1351. [PubMed: 8891338]
24. Peterson G, Barnes S. Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. Prostate 1993;22:335–345. [PubMed: 8497428]
25. Li Y, Sarkar FH. Inhibition of nuclear factor kappaB activation in PC3 cells by genistein is mediated via Akt signaling pathway. Clin Cancer Res 2002;8:2369–2377. [PubMed: 12114442]
26. Fang MZ, Chen D, Sun Y, Jin Z, Christman JK, Yang CS. Reversal of hypermethylation and reactivation of p16INK4a, RARbeta, and MGMT genes by genistein and other isoflavones from soy. Clin Cancer Res 2005;11:7033–7041. [PubMed: 16203797]
27. Cabanes A, Wang M, Olivo S, DeAssis S, Gustafsson JA, Khan G, et al. Prepubertal estradiol and genistein exposures up-regulate BRCA1 mRNA and reduce mammary tumorigenesis. Carcinogenesis 2004;25:741–748. [PubMed: 14729590]
28. Constantinou A, Kiguchi K, Huberman E. Induction of differentiation and DNA strand breakage in human HL-60 and K-562 leukemia cells by genistein. Cancer Res 1990;50:2618–2624. [PubMed: 2158395]
29. Buckley AR, Buckley DJ, Gout PW, Liang H, Rao YP, Blake MJ. Inhibition by genistein of prolactin-induced Nb2 lymphoma cell mitogenesis. Mol Cell Endocrinol 1993;98:17–25. [PubMed: 8143910]
30. Matsukawa Y, Marui N, Sakai T, Satomi Y, Yoshida M, Matsumoto K, et al. Genistein arrests cell cycle progression at G2-M. Cancer Res 1993;53:1328–1331. [PubMed: 8443813]
31. Pagliacci MC, Smacchia M, Migliorati G, Grignani F, Riccardi C, Nicoletti I. Growth-inhibitory effects of the natural phyto-oestrogen genistein in MCF-7 human breast cancer cells. Eur J Cancer 1994;30A:1675–1682. [PubMed: 7833143]
32. Casagrande F, Darbon JM. p21CIP1 is dispensable for the G2 arrest caused by genistein in human melanoma cells. Exp Cell Res 2000;258:101–108. [PubMed: 10912792]
33. Davis JN, Singh B, Bhuiyan M, Sarkar FH. Genistein-induced upregulation of p21WAF1, downregulation of cyclin B, and induction of apoptosis in prostate cancer cells. Nutr Cancer 1998;32:123–131. [PubMed: 10050261]
34. Lian F, Bhuiyan M, Li YW, Wall N, Kraut M, Sarkar FH. Genistein-induced G2-M arrest, p21WAF1 upregulation, and apoptosis in a non-small-cell lung cancer cell line. Nutr Cancer 1998;31:184–191. [PubMed: 9795970]
35. Upadhyay S, Neburi M, Chinni SR, Alhasan S, Miller F, Sarkar FH. Differential sensitivity of normal and malignant breast epithelial cells to genistein is partly mediated by p21(WAF1). Clin Cancer Res 2001;7:1782–1789. [PubMed: 11410520]
36. Kuzumaki T, Kobayashi T, Ishikawa K. Genistein induces p21(Cip1/WAF1) expression and blocks the G1 to S phase transition in mouse fibroblast and melanoma cells. Biochem Biophys Res Commun 1998;251:291–295. [PubMed: 9790949]
37. Li Y, Upadhyay S, Bhuiyan M, Sarkar FH. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. Oncogene 1999;18:3166–3172. [PubMed: 10340389]
38. Li Y, Bhuiyan M, Sarkar FH. Induction of apoptosis and inhibition of c-erbB-2 in MDA-MB-435 cells by genistein. Int J Oncol 1999;15:525–533. [PubMed: 10427135]
39. Alhasan SA, Pietrasczkiwicz H, Alonso MD, Ensley J, Sarkar FH. Genistein-induced cell cycle arrest and apoptosis in a head and neck squamous cell carcinoma cell line. Nutr Cancer 1999;34:12–19. [PubMed: 10453436]

40. Touny LH, Banerjee PP. Identification of both Myt-1 and Wee-1 as necessary mediators of the p21-independent inactivation of the cdc-2/cyclin B1 complex and growth inhibition of TRAMP cancer cells by genistein. Prostate 2006;66:1542–1555. [PubMed: 16924665]
41. Banerjee S, Zhang Y, Wang Z, Che M, Chiao PJ, Abbruzzese JL, et al. In vitro and in vivo molecular evidence of genistein action in augmenting the efficacy of cisplatin in pancreatic cancer. Int J Cancer 2007;120:906–917. [PubMed: 17131310]
42. Banerjee S, Zhang Y, Ali S, Bhuiyan M, Wang Z, Chiao PJ, et al. Molecular evidence for increased antitumor activity of gemcitabine by genistein in vitro and in vivo using an orthotopic model of pancreatic cancer. Cancer Res 2005;65:9064–9072. [PubMed: 16204081]
43. Spinozzi F, Pagliacci MC, Migliorati G, Moraca R, Grignani F, Riccardi C, et al. The natural tyrosine kinase inhibitor genistein produces cell cycle arrest and apoptosis in Jurkat T-leukemia cells. Leuk Res 1994;18:431–439. [PubMed: 8207961]
44. Kyle E, Neckers L, Takimoto C, Curt G, Bergan R. Genistein-induced apoptosis of prostate cancer cells is preceded by a specific decrease in focal adhesion kinase activity. Mol Pharmacol 1997;51:193–200. [PubMed: 9203623]
45. Moiseeva EP, Almeida GM, Jones GD, Manson MM. Extended treatment with physiologic concentrations of dietary phytochemicals results in altered gene expression, reduced growth, and apoptosis of cancer cells. Mol Cancer Ther 2007;6:3071–3079. [PubMed: 18025290]
46. Tophkhane C, Yang S, Bales W, Archer L, Osunkoya A, Thor AD, et al. Bcl-2 overexpression sensitizes MCF-7 cells to genistein by multiple mechanisms. Int J Oncol 2007;31:867–874. [PubMed: 17786319]
47. Katdare M, Osborne M, Telang NT. Soy isoflavone genistein modulates cell cycle progression and induces apoptosis in HER-2/neu oncogene expressing human breast epithelial cells. Int J Oncol 2002;21:809–815. [PubMed: 12239620]
48. Kazi A, Daniel KG, Smith DM, Kumar NB, Dou QP. Inhibition of the proteasome activity, a novel mechanism associated with the tumor cell apoptosis-inducing ability of genistein. Biochem Pharmacol 2003;66:965–976. [PubMed: 12963483]
49. Yeh TC, Chiang PC, Li TK, Hsu JL, Lin CJ, Wang SW, et al. Genistein induces apoptosis in human hepatocellular carcinomas via interaction of endoplasmic reticulum stress and mitochondrial insult. Biochem Pharmacol 2007;73:782–792. [PubMed: 17188247]
50. Davis JN, Kucuk O, Sarkar FH. Genistein inhibits NF-kappa B activation in prostate cancer cells. Nutr Cancer 1999;35:167–174. [PubMed: 10693171]
51. Alhasan SA, Ensley JF, Sarkar FH. Genistein induced molecular changes in a squamous cell carcinoma of the head and neck cell line. Int J Oncol 2000;16:333–338. [PubMed: 10639578]
52. Natarajan K, Manna SK, Chaturvedi MM, Aggarwal BB. Protein tyrosine kinase inhibitors block tumor necrosis factor-induced activation of nuclear factor-kappaB, degradation of IkappaBalpha, nuclear translocation of p65, and subsequent gene expression. Arch Biochem Biophys 1998;352:59–70. [PubMed: 9521814]
53. Davis JN, Kucuk O, Djuric Z, Sarkar FH. Soy isoflavone supplementation in healthy men prevents NF-kappa B activation by TNF-alpha in blood lymphocytes. Free Radic Biol Med 2001;30:1293–1302. [PubMed: 11368927]
54. Karin M, Delhase M. The I kappa B kinase (IKK) and NF-kappa B: key elements of proinflammatory signalling. Semin Immunol 2000;12:85–98. [PubMed: 10723801]
55. Lee FS, Peters RT, Dang LC, Maniatis T. MEKK1 activates both IkappaB kinase alpha and IkappaB kinase beta. Proc Natl Acad Sci U S A 1998;95:9319–9324. [PubMed: 9689078]
56. Sarkar FH, Li Y. Using chemopreventive agents to enhance the efficacy of cancer therapy. Cancer Res 2006;66:3347–3350. [PubMed: 16585150]
57. Mohammad RM, Banerjee S, Li Y, Aboukameel A, Kucuk O, Sarkar FH. Cisplatin-induced antitumor activity is potentiated by the soy isoflavone genistein in BxPC-3 pancreatic tumor xenografts. Cancer 2006;106:1260–1268. [PubMed: 16475211]
58. Li Y, Ellis KL, Ali S, El-Rayes BF, Nedeljkovic-Kurepa A, Kucuk O, et al. Apoptosis-inducing effect of chemotherapeutic agents is potentiated by soy isoflavone genistein, a natural inhibitor of NF-kappaB in BxPC-3 pancreatic cancer cell line. Pancreas 2004;28:e90–95. [PubMed: 15097869]

59. Li Y, Kucuk O, Hussain M, Abrams J, Cher ML, Sarkar FH. Antitumor and antimetastatic activities of docetaxel are enhanced by genistein through regulation of osteoprotegerin/receptor activator of nuclear factor-kappaB (RANK)/RANK ligand/MMP-9 signaling in prostate cancer. Cancer Res 2006;66:4816–4825. [PubMed: 16651437]
60. Romashkova JA, Makarov SS. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. Nature 1999;401:86–90. [PubMed: 10485711]
61. Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature 1999;401:82–85. [PubMed: 10485710]
62. Yang CH, Murti A, Pfeffer SR, Kim JG, Donner DB, Pfeffer LM. Interferon alpha/beta promotes cell survival by activating nuclear factor kappa B through phosphatidylinositol 3-kinase and Akt. J Biol Chem 2001;276:13756–13761. [PubMed: 11278812]
63. Stoica GE, Franke TF, Wellstein A, Czubayko F, List HJ, Reiter R, et al. Estradiol rapidly activates Akt via the ErbB2 signaling pathway. Mol Endocrinol 2003;17:818–830. [PubMed: 12554767]
64. Stoica GE, Franke TF, Wellstein A, Morgan E, Czubayko F, List HJ, et al. Heregulin-beta1 regulates the estrogen receptor-alpha gene expression and activity via the ErbB2/PI 3-K/Akt pathway. Oncogene 2003;22:2073–2087. [PubMed: 12687010]
65. El Touny LH, Banerjee PP. Akt GSK-3 pathway as a target in genistein-induced inhibition of TRAMP prostate cancer progression toward a poorly differentiated phenotype. Carcinogenesis 2007;28:1710–1717. [PubMed: 17468512]
66. Montgomery JS, Price DK, Figg WD. The androgen receptor gene and its influence on the development and progression of prostate cancer. J Pathol 2001;195:138–146. [PubMed: 11592091]
67. Kupelian P, Katcher J, Levin H, Zippe C, Klein E. Correlation of clinical and pathologic factors with rising prostate-specific antigen profiles after radical prostatectomy alone for clinically localized prostate cancer. Urology 1996;48:249–260. [PubMed: 8753737]
68. Luke MC, Coffey DS. Human androgen receptor binding to the androgen response element of prostate specific antigen. J Androl 1994;15:41–51. [PubMed: 7514587]
69. Sato N, Gleave ME, Bruchovsky N, Rennie PS, Goldenberg SL, Lange PH, et al. Intermittent androgen suppression delays progression to androgen-independent regulation of prostate-specific antigen gene in the LNCaP prostate tumour model. J Steroid Biochem Mol Biol 1996;58:139–146. [PubMed: 8809195]
70. Davis JN, Muqim N, Bhuiyan M, Kucuk O, Pienta KJ, Sarkar FH. Inhibition of prostate specific antigen expression by genistein in prostate cancer cells. Int J Oncol 2000;16:1091–1097. [PubMed: 10811979]
71. Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, et al. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. Embo J 1999;18:4608–4618. [PubMed: 10469641]
72. Seger R, Krebs EG. The MAPK signaling cascade. Faseb J 1995;9:726–735. [PubMed: 7601337]
73. Sebolt-Leopold JS. Development of anticancer drugs targeting the MAP kinase pathway. Oncogene 2000;19:6594–6599. [PubMed: 11426644]
74. Kong AN, Yu R, Hebbar V, Chen C, Owuor E, Hu R, et al. Signal transduction events elicited by cancer prevention compounds. Mutat Res 2001;480–481:231–241.
75. Huang X, Chen S, Xu L, Liu Y, Deb DK, Platanias LC, et al. Genistein inhibits p38 map kinase activation, matrix metalloproteinase type 2, and cell invasion in human prostate epithelial cells. Cancer Res 2005;65:3470–3478. [PubMed: 15833883]
76. Kim EK, Kwon KB, Song MY, Seo SW, Park SJ, Ka SO, et al. Genistein protects pancreatic beta cells against cytokine-mediated toxicity. Mol Cell Endocrinol 2007;278:18–28. [PubMed: 17881116]
77. Lee MW, Bach JH, Lee HJ, Lee DY, Joo WS, Kim YS, et al. The activation of ERK1/2 via a tyrosine kinase pathway attenuates trail-induced apoptosis in HeLa cells. Cancer Invest 2005;23:586–592. [PubMed: 16305985]
78. Wei H, Wei L, Frenkel K, Bowen R, Barnes S. Inhibition of tumor promoter-induced hydrogen peroxide formation in vitro and in vivo by genistein. Nutr Cancer 1993;20:1–12. [PubMed: 8415125]

79. Rotondo S, Krauze-Brzosko K, Manarini S, Martelli N, Pecce R, Evangelista V, et al. Inhibition by soya isoflavones of human polymorphonuclear leukocyte function: possible relevance for the beneficial effects of soya intake. Br J Nutr 2007:1–8.
80. Hernandez-Montes E, Pollard SE, Vauzour D, Jofre-Montseny L, Rota C, Rimbach G, et al. Activation of glutathione peroxidase via Nrf1 mediates genistein's protection against oxidative endothelial cell injury. Biochem Biophys Res Commun 2006;346:851–859. [PubMed: 16780800]
81. Kameoka S, Leavitt P, Chang C, Kuo SM. Expression of antioxidant proteins in human intestinal Caco-2 cells treated with dietary flavonoids. Cancer Lett 1999;146:161–167. [PubMed: 10656621]
82. Dudek EJ, Shang F, Taylor A. H(2)O(2)-mediated oxidative stress activates NF-kappa B in lens epithelial cells. Free Radic Biol Med 2001;31:651–658. [PubMed: 11522450]
83. Lau TY, Leung LK. Soya isoflavones suppress phorbol 12-myristate 13-acetate-induced COX-2 expression in MCF-7 cells. Br J Nutr 2006;96:169–176. [PubMed: 16870006]
84. Hwang JT, Ha J, Park OJ. Combination of 5-fluorouracil and genistein induces apoptosis synergistically in chemo-resistant cancer cells through the modulation of AMPK and COX-2 signaling pathways. Biochem Biophys Res Commun 2005;332:433–440. [PubMed: 15896711]
85. Raffoul JJ, Banerjee S, Singh-Gupta V, Knoll ZE, Fite A, Zhang H, et al. Down-regulation of apurinic/apyrimidinic endonuclease 1/redox factor-1 expression by soy isoflavones enhances prostate cancer radiotherapy in vitro and in vivo. Cancer Res 2007;67:2141–2149. [PubMed: 17332344]
86. Su Y, Simmen FA, Xiao R, Simmen RC. Expression profiling of rat mammary epithelial cells reveals candidate signaling pathways in dietary protection from mammary tumors. Physiol Genomics 2007;30:8–16. [PubMed: 17341692]
87. Hawrylewicz EJ, Zapata JJ, Blair WH. Soy and experimental cancer: animal studies. J Nutr 1995;125:698S–708S. [PubMed: 7884554]
88. Ravindranath MH, Muthugounder S, Presser N, Viswanathan S. Anticancer therapeutic potential of soy isoflavone, genistein. Adv Exp Med Biol 2004;546:121–165. [PubMed: 15584372]
89. Fritz WA, Coward L, Wang J, Lamartiniere CA. Dietary genistein: perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat. Carcinogenesis 1998;19:2151–2158. [PubMed: 9886571]
90. Sharma OP, Adlercreutz H, Strandberg JD, Zirkin BR, Coffey DS, Ewing LL. Soy of dietary source plays a preventive role against the pathogenesis of prostatitis in rats. J Steroid Biochem Mol Biol 1992;43:557–564. [PubMed: 1419891]
91. Onozawa M, Kawamori T, Baba M, Fukuda K, Toda T, Sato H, et al. Effects of a soybean isoflavone mixture on carcinogenesis in prostate and seminal vesicles of F344 rats. Jpn J Cancer Res 1999;90:393–398. [PubMed: 10363576]
92. Tanaka T, Kohno H, Tanino M, Yanaida Y. Inhibitory effects of estrogenic compounds, 4-nonylphenol and genistein, on 7,12-dimethylbenz[a]anthracene-induced ovarian carcinogenesis in rats. Ecotoxicol Environ Saf 2002;52:38–45. [PubMed: 12051806]
93. Landstrom M, Zhang JX, Hallmans G, Aman P, Bergh A, Damber JE, et al. Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats. Prostate 1998;36:151–161. [PubMed: 9687986]
94. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. J Nutr 1999;129:1628–1635. [PubMed: 10460196]
95. Li D, Yee JA, McGuire MH, Murphy PA, Yan L. Soybean isoflavones reduce experimental metastasis in mice. J Nutr 1999;129:1075–1078. [PubMed: 10222402]
96. Lamartiniere CA, Moore JB, Brown NM, Thompson R, Hardin MJ, Barnes S. Genistein suppresses mammary cancer in rats. Carcinogenesis 1995;16:2833–2840. [PubMed: 7586206]
97. Uckun FM, Evans WE, Forsyth CJ, Waddick KG, Ahlgren LT, Chelstrom LM, et al. Biotherapy of B-cell precursor leukemia by targeting genistein to CD19-associated tyrosine kinases. Science 1995;267:886–891. [PubMed: 7531365]
98. Singh AV, Franke AA, Blackburn GL, Zhou JR. Soy phytochemicals prevent orthotopic growth and metastasis of bladder cancer in mice by alterations of cancer cell proliferation and apoptosis and tumor angiogenesis. Cancer Res 2006;66:1851–1858. [PubMed: 16452247]

99. Wei H, Saladi R, Lu Y, Wang Y, Palep SR, Moore J, et al. Isoflavone genistein: photoprotection and clinical implications in dermatology. J Nutr 2003;133:3811S–3819S. [PubMed: 14608119]
100. Shyong EQ, Lu Y, Lazinsky A, Saladi RN, Phelps RG, Austin LM, et al. Effects of the isoflavone 4′,5,7-trihydroxyisoflavone (genistein) on psoralen plus ultraviolet A radiation (PUVA)-induced photodamage. Carcinogenesis 2002;23:317–321. [PubMed: 11872639]
101. Begum M, Tashiro H, Katabuchi H, Suzuki A, Kurman RJ, Okamura H. Neonatal estrogenic exposure suppresses PTEN-related endometrial carcinogenesis in recombinant mice. Lab Invest 2006;86:286–296. [PubMed: 16402032]
102. Tatsuta M, Iishi H, Baba M, Yano H, Uehara H, Nakaizumi A. Attenuation by genistein of sodium-chloride-enhanced gastric carcinogenesis induced by N-methyl-N′-nitro-N-nitrosoguanidine in Wistar rats. Int J Cancer 1999;80:396–399. [PubMed: 9935180]
103. Tan M, Yao J, Yu D. Overexpression of the c-erbB-2 gene enhanced intrinsic metastasis potential in human breast cancer cells without increasing their transformation abilities. Cancer Res 1997;57:1199–1205. [PubMed: 9067293]
104. Fotsis T, Pepper M, Adlercreutz H, Hase T, Montesano R, Schweigerer L. Genistein, a dietary ingested isoflavonoid, inhibits cell proliferation and in vitro angiogenesis. J Nutr 1995;125:790S–797S. [PubMed: 7533831]
105. Owen JL, Torroella-Kouri M, Iragavarapu-Charyulu V. Molecular events involved in the increased expression of matrix metalloproteinase-9 by T lymphocytes of mammary tumor-bearing mice. Int J Mol Med 2008;21:125–134. [PubMed: 18097625]
106. Li Y, Sarkar FH. Down-regulation of invasion and angiogenesis-related genes identified by cDNA microarray analysis of PC3 prostate cancer cells treated with genistein. Cancer Lett 2002;186:157–164. [PubMed: 12213285]
107. Xu L, Bergan RC. Genistein inhibits matrix metalloproteinase type 2 activation and prostate cancer cell invasion by blocking the transforming growth factor beta-mediated activation of mitogen-activated protein kinase-activated protein kinase 2-27-kDa heat shock protein pathway. Mol Pharmacol 2006;70:869–877. [PubMed: 16772519]
108. Lee WY, Huang SC, Tzeng CC, Chang TL, Hsu KF. Alterations of metastasis-related genes identified using an oligonucleotide microarray of genistein-treated HCC1395 breast cancer cells. Nutr Cancer 2007;58:239–246. [PubMed: 17640171]
109. Li Y, Che M, Bhagat S, Ellis KL, Kucuk O, Doerge DR, et al. Regulation of gene expression and inhibition of experimental prostate cancer bone metastasis by dietary genistein. Neoplasia 2004;6:354–363. [PubMed: 15256057]
110. Iwasaki T, Mukai M, Tsujimura T, Tatsuta M, Nakamura H, Terada N, et al. Ipriflavone inhibits osteolytic bone metastasis of human breast cancer cells in a nude mouse model. Int J Cancer 2002;100:381–387. [PubMed: 12115517]
111. Zhou JR, Yu L, Zhong Y, Nassr RL, Franke AA, Gaston SM, et al. Inhibition of orthotopic growth and metastasis of androgen-sensitive human prostate tumors in mice by bioactive soybean components. Prostate 2002;53:143–153. [PubMed: 12242729]
112. Miekus K, Madeja Z. Genistein inhibits the contact-stimulated migration of prostate cancer cells. Cell Mol Biol Lett. 2007In press
113. Craft CS, Xu L, Romero D, Vary CP, Bergan RC. Genistein induces phenotypic reversion of endoglin deficiency in human prostate cancer cells. Mol Pharmacol 2008;73:235–242. [PubMed: 17951357]
114. El Touny LH, Banerjee PP. Genistein induces the metastasis suppressor kangai-1 which mediates its anti-invasive effects in TRAMP cancer cells. Biochemical and biophysical research communications 2007;361:169–175. [PubMed: 17658479]
115. Roberts AB, Flanders KC, Heine UI, Jakowlew S, Kondaiah P, Kim SJ, et al. Transforming growth factor-beta: multifunctional regulator of differentiation and development. Philos Trans R Soc Lond B Biol Sci 1990;327:145–154. [PubMed: 1969655]
116. Kim H, Peterson TG, Barnes S. Mechanisms of action of the soy isoflavone genistein: emerging role for its effects via transforming growth factor beta signaling pathways. Am J Clin Nutr 1998;68:1418S–1425S. [PubMed: 9848510]

117. Su SJ, Yeh TM, Chuang WJ, Ho CL, Chang KL, Cheng HL, et al. The novel targets for anti-angiogenesis of genistein on human cancer cells. Biochem Pharmacol 2005;69:307–318. [PubMed: 15627483]
118. Guo Y, Wang S, Hoot DR, Clinton SK. Suppression of VEGF-mediated autocrine and paracrine interactions between prostate cancer cells and vascular endothelial cells by soy isoflavones. J Nutr Biochem 2007;18:408–417. [PubMed: 17142033]
119. Mohammad RM, Al-Katib A, Aboukameel A, Doerge DR, Sarkar F, Kucuk O. Genistein sensitizes diffuse large cell lymphoma to CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy. Mol Cancer Ther 2003;2:1361–1368. [PubMed: 14707277]
120. Li Y, Ahmed F, Ali S, Philip PA, Kucuk O, Sarkar FH. Inactivation of nuclear factor kappaB by soy isoflavone genistein contributes to increased apoptosis induced by chemotherapeutic agents in human cancer cells. Cancer Res 2005;65:6934–6942. [PubMed: 16061678]
121. El-Rayes BF, Ali S, Ali IF, Philip PA, Abbruzzese J, Sarkar FH. Potentiation of the effect of erlotinib by genistein in pancreatic cancer: the role of Akt and nuclear factor-kappaB. Cancer Res 2006;66:10553–10559. [PubMed: 17079479]
122. Mai Z, Blackburn GL, Zhou JR. Genistein sensitizes inhibitory effect of tamoxifen on the growth of estrogen receptor-positive and HER2-overexpressing human breast cancer cells. Mol Carcinog 2007;46:534–542. [PubMed: 17295235]
123. Vinall RL, Hwa K, Ghosh P, Pan CX, Lara PN Jr, de Vere White RW. Combination treatment of prostate cancer cell lines with bioactive soy isoflavones and perifosine causes increased growth arrest and/or apoptosis. Clin Cancer Res 2007;13:6204–6216. [PubMed: 17947488]
124. Shen J, Tai YC, Zhou J, Stephen Wong CH, Cheang PT, Fred Wong WS, et al. Synergistic antileukemia effect of genistein and chemotherapy in mouse xenograft model and potential mechanism through MAPK signaling. Exp Hematol 2007;35:75–83. [PubMed: 17198876]
125. Satoh H, Nishikawa K, Suzuki K, Asano R, Virgona N, Ichikawa T, et al. Genistein, a soy isoflavone, enhances necrotic-like cell death in a breast cancer cell treated with a chemotherapeutic agent. Res Commun Mol Pathol Pharmacol 2003;113–114:149–158.
126. Tanos V, Brzezinski A, Drize O, Strauss N, Peretz T. Synergistic inhibitory effects of genistein and tamoxifen on human dysplastic and malignant epithelial breast cells in vitro. Eur J Obstet Gynecol Reprod Biol 2002;102:188–194. [PubMed: 11950489]
127. Lee R, Kim YJ, Lee YJ, Chung HW. The selective effect of genistein on the toxicity of bleomycin in normal lymphocytes and HL-60 cells. Toxicology 2004;195:87–95. [PubMed: 14751666]
128. Khoshyomn S, Manske GC, Lew SM, Wald SL, Penar PL. Synergistic action of genistein and cisplatin on growth inhibition and cytotoxicity of human medulloblastoma cells. Pediatr Neurosurg 2000;33:123–131. [PubMed: 11096359]
129. Novak-Hofer I. The L1 cell adhesion molecule as a target for radioimmunotherapy. Cancer Biother Radiopharm 2007;22:175–184. [PubMed: 17600464]
130. Nozawa F, Itami A, Saruc M, Kim M, Standop J, Picha KS, et al. The combination of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) and Genistein is effective in inhibiting pancreatic cancer growth. Pancreas 2004;29:45–52. [PubMed: 15211111]
131. Jin CY, Park C, Cheong J, Choi BT, Lee TH, Lee JD, et al. Genistein sensitizes TRAIL-resistant human gastric adenocarcinoma AGS cells through activation of caspase-3. Cancer Lett 2007;257:56–64. [PubMed: 17689858]
132. Hillman GG, Forman JD, Kucuk O, Yudelev M, Maughan RL, Rubio J, et al. Genistein potentiates the radiation effect on prostate carcinoma cells. Clin Cancer Res 2001;7:382–390. [PubMed: 11234894]
133. Hillman GG, Wang Y, Che M, Raffoul JJ, Yudelev M, Kucuk O, et al. Progression of renal cell carcinoma is inhibited by genistein and radiation in an orthotopic model. BMC Cancer 2007;7:4. [PubMed: 17212824]
134. Akimoto T, Nonaka T, Ishikawa H, Sakurai H, Saitoh JI, Takahashi T, et al. Genistein, a tyrosine kinase inhibitor, enhanced radiosensitivity in human esophageal cancer cell lines in vitro: possible involvement of inhibition of survival signal transduction pathways. Int J Radiat Oncol Biol Phys 2001;50:195–201. [PubMed: 11316564]

135. Yashar CM, Spanos WJ, Taylor DD, Gercel-Taylor C. Potentiation of the radiation effect with genistein in cervical cancer cells. Gynecol Oncol 2005;99:199–205. [PubMed: 16083949]

136. Constantinou AI, Mehta R, Husband A. Phenoxodiol, a novel isoflavone derivative, inhibits dimethylbenz[a]anthracene (DMBA)-induced mammary carcinogenesis in female Sprague-Dawley rats. European Journal of Cancer 2003;39:1012–1018. [PubMed: 12706372]

137. Alvero AB, O'Malley D, Brown D, Kelly G, Garg M, Chen W, et al. Molecular mechanism of phenoxodiol-induced apoptosis in ovarian carcinoma cells. Cancer 2006;106:599–608. [PubMed: 16388521]

138. Sapi E, Alvero AB, Chen W, O'Malley D, Hao XY, Dwipoyono B, et al. Resistance of ovarian carcinoma cells to docetaxel is XIAP dependent and reversible by phenoxodiol. Oncology Research 2004;14:567–578. [PubMed: 15666998]

139. Sarkar FH, Adsule S, Padhye S, Kulkarni S, Li Y. The role of genistein and synthetic derivatives of isoflavone in cancer prevention and therapy. Mini Rev Med Chem 2006;6:401–407. [PubMed: 16613577]

140. Fu XH, Wang L, Zhao H, Xiang HL, Cao JG. Synthesis of genistein derivatives and determination of their protective effects against vascular endothelial cell damages caused by hydrogen peroxide. Bioorg Med Chem Lett. 2007

141. Li HQ, Ge HM, Chen YX, Xu C, Shi L, Ding H, et al. Synthesis and cytotoxic evaluation of a series of genistein derivatives. Chem Biodivers 2006;3:463–472. [PubMed: 17193282]

142. Fischer L, Mahoney C, Jeffcoat AR, Koch MA, Thomas BE, Valentine JL, et al. Clinical characteristics and pharmacokinetics of purified soy isoflavones: multiple-dose administration to men with prostate neoplasia. Nutrition and Cancer 2004;48:160–170. [PubMed: 15231450]

143. Miltyk W, Craciunescu CN, Fischer L, Jeffcoat RA, Koch MA, Lopaczynski W, et al. Lack of significant genotoxicity of purified soy isoflavones (genistein, daidzein, and glycitein) in 20 patients with prostate cancer. American Journal of Clinical Nutrition 2003;77:875–882. [PubMed: 12663286]

144. Kumar NB, Krischer JP, Allen K, Riccardi D, Besterman-Dahan K, Salup R, et al. A Phase II Randomized, Placebo-Controlled Clinical Trial of Purified Isoflavones in Modulating Steroid Hormones in Men Diagnosed With Localized Prostate Cancer. Nutrition and Cancer 2007;59:163–168. [PubMed: 18001210]

145. Vaishampayan U, Hussain M, Banerjee M, Seren S, Sarkar FH, Fontana J, et al. Lycopene and soy isoflavones in the treatment of prostate cancer. Nutrition and Cancer 2007;59:1–7. [PubMed: 17927495]

146. Dalais FS, Meliala A, Wattanapenpaiboon N, Frydenberg M, Suter DA, Thomson WK, et al. Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. Urology 2004;64:510–515. [PubMed: 15351581]

Table-1

Molecular targets of genistein

Apoptosis	Transcription factors	Cell cycle	Others
↑Bax	↓NF-κB	↓Cyclin B1	↓Akt
↓Bcl-2	↑Nrf1	↓Cyclin D1	↓AR
↓Bcl-xL	↑Nrf2	↑$p21^{WAF1}$	↓PSA
↑PARP	↓STAT-3	↑$p27^{KIP1}$	↓COX-2
↓Survivin	↓STAT-5	↑$p16^{INK4a}$	↓MMP-9
↓IAP	↓IGF-1R	↑Myt-1	↓MMP-2
↓XIAP	↓Ape-1/Ref	↓Wee-1	↓p38 MAPK
↑BAD	↓Wnt	↓CDK-1	↓ERK-1/2
↑Active caspases	↓vNotch-2		↑GPx
↑ER stress regulators	↓AP-1		↑kangai-1
	↓CREB		↑Endoglin
	↑GADD 153		↓RANK/RANK-L
	↓HIF-1α		↓PTEN