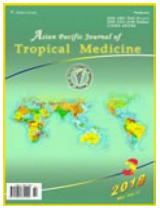

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Dietary isoflavones, the modulator of breast carcinogenesis: Current landscape and future perspectives

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ABSTRACT

Breast cancer is a frightful disease and serious concern in women around the world causing significant health care burden in both developed and developing countries. Extensive research work has shown that breast cancer provides strong resistance to chemical agents, UV radiation, and hormonal treatments. It is generally accepted that cell genetics is not the only main reason for breast cancer and genetic risk factors, for example, mutations in *BRCA1* and *BRCA2* genes constitute 5%-10% of all breast cancer rates. Other related factors include age, gender, race, ethnicity, weight, reproductive factors, exo- and endogenous hormonal exposures, oral contraceptives use, ultraviolet radiation, diet, and night work (circadian disruption). Many studies have revealed that dietary isoflavones regulate breast cancer occurrence, recurrence and prognosis. Dietary isoflavones have long been part of Asian population diet and there is a significant increase as compared to dietary isoflavones intake among other populations. Dietary isoflavones are natural phytoestrogens having both estrogenic and anti-estrogenic potentials on breast cancer cells in culture, animal models and in experimental trials. This literature survey provides a comprehensive overview on the tumor preventive and tumor promoting potentials of dietary isoflavones on breast cancer. In addition, this paper provides a literature review of dietary isoflavones and their effects on up-regulation and down-regulation of different signaling pathways, genes and proteins. Finally, future perspectives of dietary isoflavones and breast cancer researchers are also critically discussed, which will provide a deeper insight regarding the inner molecular mechanisms of action.

1. Background

Breast cancer is a serious concern at present despite of recent medical advances. Breast cancer is a frightful disease in female around the world. According to breast cancer statistics an approximately 14 000 000 new breast cancer cases and around 8 000 000 deaths are reported per year around the globe[1]. According to 2017 breast cancer statistics report, United States alone will have 255 180 new cases of breast cancer and 41 070 deaths[2]. However, breast cancer is not restricted to females only, it also affects males[3,4] and transgender individuals[5,6]. Obviously, development of novel, affordable, more potent and side effects lacking therapy is the need of hour in health care pharmacy. Results from large numbers of research studies indicated that there is a close relationship between diet and cancer rates[7-9]. Additionally, dietary factors may account for 30%-35%

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of all the cancer types[10]. Large numbers of factors are associated with increasing breast cancer risk including nutrients containing high amounts of sugar, alcohol and animal or caloric content[11,12]. These dietary isoflavones perform anticancer functions by modulating different nuclear and cellular signaling cascades, induces free radical scavenging, modulate enzymatic and hormonal activities and induces DNA damage in breast cancer cells[13-16].

There are large numbers of phytochemicals that are widely distributed in medicinal plants. Five major classes of phytoestrogens (naturally occurring plant compounds) namely stilbenes, lignans, flavonoids, isoflavonoids, and coumestans[17]. The present review article gives detailed information on the correlation between dietary soy isoflavones and breast cancer risk. Dietary isoflavones have a wide range of distribution in a number of food stuffs including soybeans, beans and lentils[18]. The leading soy isoflavones are genistein, daidzein and glycitein and are used for breast cancer treatment.

The amount of dietary isoflavonoids people use varies in different geographical regions of the world. For example, the average daily dietary isoflavone consumption of older people in Japan is around 30-50 mg[19], followed by the United States[20] and Europe[21], where it is less than 3 mg per capita. The traditional sources of isoflavones in Asian diet include miso, tofu, soy-milk and tempeh, while Western people diet contains other food sources of isoflavone such as, meat products added with soy proteins and soy-based meat derivatives[22]. A number of these second-generation soy products are highly rich in soy isoflavone content than conventional Asian soy isoflavone products[23]. Because of their diverse chemical and molecular structure, the isoflavones can bind to estrogen receptors (ER) where it either inhibits or promotes the estrogen-sensitive genes expression level[24,25].

It has been studied that the incidence of breast cancer rate is significantly lower in Asian people as compared with people in other parts around the world because of high isoflavones consumption in their regular diet[26,27]. In one more study of Verheus *et al*, findings have shown elevated plasma level of genistein which has lower down the breast cancer risk in Dutch females while consuming high level of isoflavones[28] and there was a nine-fold difference between the regular amount of dietary isoflavones intake by Chinese Americans (4 grams per day) as compared with native Chinese (36 grams per day) after consumption of this high amount of isoflavones in different experimental trials[29]. It has been demonstrated that intake of high soy isoflavones during early ages may lower down the risk of having breast cancer and that risk may be further lowered down through regular soy diet intake in older age[30,31].

The phytoestrogen is attracting more attention because they offer a safer and more effective alternative as compared with hormonal replacement treatment in postmenopausal women[32]. Research has also proved that s-equol, (daidzein metabolite), was noticed to mitigate menopausal symptoms[33]. In the US, women have approximately 14% breast cancer probability during their lifetime as they are now moving towards relatively having low-cost life style and taking isoflavone rich diet to combat with this global menace[34]. These soy isoflavones can also be used as a potential candidate for the treatments of breast cancer as they have strong anti-neoplastic functions. The soy isoflavones can prevent the occurrence of breast cancer by inhibiting enzymes essential for replicating DNA, metastasis, disabling growth factors such as VEGF that promote angiogenesis and activate immune system[35,25]. Dietary isoflavones functions are illustrated in Figure 1.

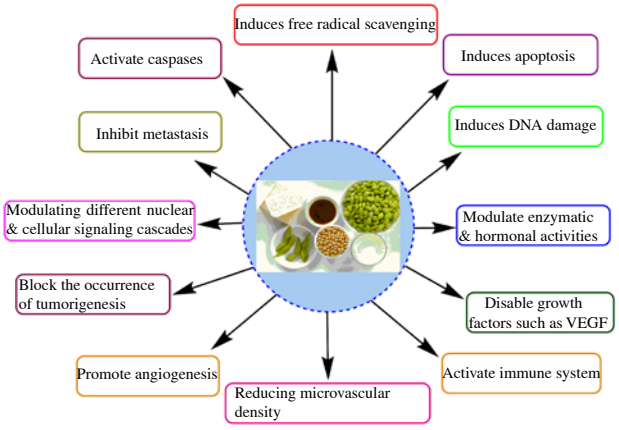


Figure 1. Dietary isoflavones while performing different functions at nuclear and cellular levels.

The detailed process by which these dietary isoflavones modulate breast cancer is not completely understood and need further research work to better evaluate this complex mechanism. This review article will focus on the advantages (protective effects) and disadvantages (harmful side effects) of these phytoestrogens and give deeper overview on their future perspectives.

2. Dietary isoflavones as a potential source for breast cancer prevention

Estrogen hormone induces breast cancer initiation, promotion, progression and interventions. As a result, these dietary isoflavones are consumed to modify, stop or lower down estrogen hormone production and result in favorable prognoses of breast cancer patients. Several research studies have been conducted on these dietary isoflavones regarding breast cancer. Yamamoto and his team conducted population-based prospective study and came up with the conclusion, that repeated dietary isoflavones intake is inversely proportional to lower risk of breast cancer[36]. It has been researched, that dietary isoflavones do not have estrogenic effects on human's tissues and decided that these soy foods may provide safer alternative for breast cancer patients survivors[37]. These research findings also concluded that these dietary isoflavones are chemopreventive and

chemotherapeutic and prevent the occurrence of breast cancer.

It has been researched that soy isoflavones inhibited breast cancer cells in both cases: *in vitro* and *in vivo* studies. Genistein inhibits migration, growth, and invasion of breast cancer cells in nude mice implanted with breast cancer cell lines such as MCF-7 and MDA-MB-231 breast cancer[38]. These isoflavones can also inhibit breast cancer by down-regulating the expression level of matrixmettaloproteinase-9 (MMP-9) enzymes which play a crucial role in breast cancer control[39]. Moreover, daidzein's also have potent anti-proliferative effects via inhibiting cytokines, up-regulate Bax/Bcl-2 ratio via inducing apoptosis, increase antioxidant level and up-regulate cyclin dependent kinases (Cdks)[40,41]. Additionally, daidzein's isoflavone also has the potential to suppress the invasion and migration of MDA-MB-231 cells that is regulated through NF-kappa B dependent signaling cascade[42]. Moreover, isoflavones can also have the potential to stop angiogenesis by reducing microvascular density in terms of number and length, decrease circulating levels of VEGF and increase endostatin levels, thus playing a vital role in regulating breast cancer cells[43].

Natural phytoestrogen genistein has been found to exert potential estrogen mimetic role having similar structure to that of endocrine hormone[44]. Large numbers of breast cancer patients have ER-positive breast cancer[45]. This is one more important cause of why a clear understanding of how isoflavones binding to ER may be used to develop new therapeutic strategies. Genistein causes apoptosis in a wide range of cancers with different status of ER, proposing that it is a promising alternative for breast cancer treatment. For instance, genistein activates caspase-3 enzyme by inducing apoptosis in ER (+) and ER (–) breast cancer cell lines[45,46]. Furthermore, it has been demonstrated in recent research studies that genistein induces apoptosis in breast cancer cells by down-regulating the expression levels of miR-155 responsible for causing breast cancer[47]. All these research studies concluded the growth inhibitory effects of genistein by activating different apoptotic pathways at nuclear and cellular level. Detailed information about these dietary isoflavones and their inhibitory role in different molecular pathways are given in Figure 2. Genistein along with adriamycin when applied resulted in necrosis in breast cancer cells by deactivating HER-2 receptor[48].

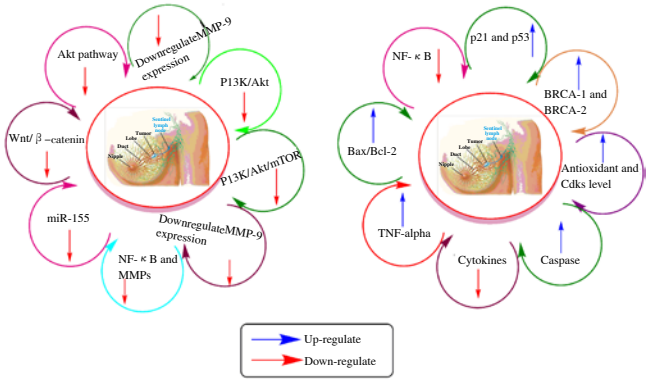


Figure 2. Molecular mechanisms of inhibitary effects of dietary isoflavones on breast cancer.

These dietary isoflavones either up-regulate or down-regulate these signaling pathways in order to arrest breast cancer. Up-regulation is shown with blue color arrow while down-regulation is shown with red color arrow.

Genistein have also played a very significant role via suppressing the invasion and metastatic effects on different breast cancer cell lines, such as MCF-7 and MDA-MB-231 by down-regulating the expression of many MMPs[49], modulating different molecular pathways involved in expression of many genes and their protein products, such as Bax, Akt, NF- κ B, Bcl-2 and so on[50]. Breast cancer regulated genes have played an important role in breast cancer regulation, for example, *BRCA–1* and *BRCA–2* have been examined as markers in different breast cancer therapies and genistein has shown apoptotic potential in BRCA-1 wild type and BRCA mutant cancer cells resulting in breast cancer treatment[51].

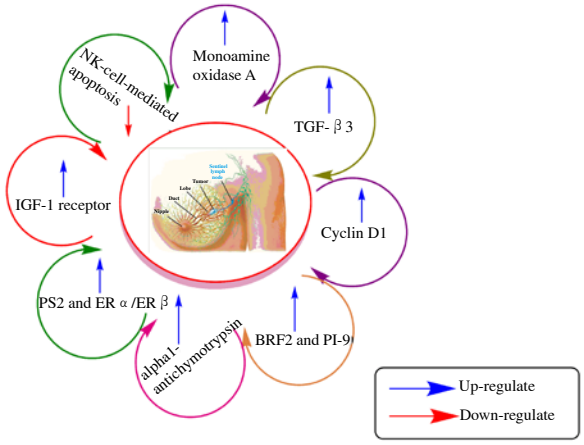
It has been investigated that genistein accelerates tumor-necrosis factor α and *p53* gene expression in breast cancer cells and has shown promising effects on breast cancer risks when these phytochemicals are consumed at young ages[51,52]. Similarly, Gotoh *et al*. in his study, fed animals with a 10% miso diet (an isoflavone) has shown a high decrease in mammary cancer per mouse[53]. Another study has also examined that soy intake in puberty also noticed a decrease in breast cancer implanted in animal models which were nourished with soy having normal concentration of dietary isoflavones[54]. Shu *et al*, in his study, he observed that intake of soy isoflavones has lowered down the breast cancer risk in individuals at their young age than those without soy diet[55]. They came up with a conclusion that soy intake around the age of 13 to 15 years caused reduced breast cancer risk later than those did not consumed soy-riched diet in earlier ages[55]. The data extracted from Wu *et al*. also supported the fact that there is an inverse relationship among dietary isoflavones intake and breast cancer risk, supporting the notion that the higher dietary isoflavones consumption is, the lower breast cancer occurrence will be[30].

3. Dietary isoflavones as a potential source for breast cancer promotion

Dietary isoflavones not only cause breast cancer prevention, but they also cause breast cancer promotion by applying their estrogenic effects, which has caused fear and nervousness among the scientific community when patients are recommended these agents as possible therapeutic option. This research work is not baseless and there are large numbers of scientific evidences from researches to back up this notion. According to Allred and the scientific findings of his team, different genistein concentrations caused neoplastic growth and tumor in mice implanted with MCF-7 xenograft tumor and promoted breast cancer[56]. In addition, Hsieh *et al*. investigated the effect of 100 nM genistein, where it triggered invasion and proliferation in MCF-7 cells in *in vitro* and in mice bearing MCF-7 tumor in mammary glands[57]. These results propose that the estrogenic effects of genistein are not only restricted to cells in culture, but they also show effects on normal healthy breast tissue. In contrast, Ju *et al*. investigated that the intake of dietary daidzein, also has the ability to stimulate or promote growth of athymic mice implanted with MCF-7 breast cancer cells[58]. Similarly, Johnson *et al*. also showed that daidzein caused promotion, invasion, and proliferation in breast cancer cells and these daidzin's tumor promoting effects also continue through ER[59]. Additionally, Isoda *et al*. suggested that

daidzein attach to ER and this attachment was stopped by tamoxifen treatment in the same study[60].

How does genistein perform its functions through the ER? Many scientific evidences indicated that genistein tumor effects occur through its unique binding pattern with ER α [61,62]. Genistein apparently binds to ER and trigger estrogen-dependent genes for regulating breast cancer, for example, monoamine oxidase A (promotes metastasis), TGF- β 3 and α 1-antichymotrypsin in *in vitro* studies[63]. The 1-antichymotrypsin is produced by MCF-7 cells *in vitro* and is considered to have a promising function in the proteolytic degradation and results in inducing metastasis[64]. Dietary isoflavones and their stimulatory effects on different molecular mechanisms are given in Figure 3.



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Moreover, genistein consists of ligand binding domain that has similarity in its chemical structure compared to 17- β estradiol. As a result, it binds very efficiently to nuclear and cellular factors and performs its anti-breast cancer function[65]. Genistein also performs its function to negate the chemotherapeutic potential of tamoxifen. As mentioned previously, proliferation and invasion of mammary gland tumor was observed in a nude mice nourished with a low dosage of genistein and was treated with tamoxifen[13]. Similarly, Limer observed that genistein treatment showed invasive and cell proliferative properties in tamoxifen-responsive cells (MCF-7 cells)[66]. Genistein modulates ER and describes how it employs these functions such as proliferating ER (+) cells, inhibiting the effects of tamoxifen and activating genes responsible for cell cycle regulation[13,67]. Cells treated with genistein isoflavones are capable of avoiding apoptosis. Jiang *et al*. found that genistein up-regulated proteinase inhibitor 9, mRNA and protein levels and proteinase inhibitor 9 (Figure 3) and inhibited apoptosis in MCF-7 cells through natural killer cells[68]. The epigenetic potential of isoflavones has been studied on ER α expression. Li *et al*. observed that genistein treatment increased ER α mRNA and protein expression level in an ER α -negative cell line (MDA-MB-231)[69]. Likewise, Berner *et al*. suggested that genistein have significant effects on hypomethylation of the ER α promoter in colon cancer cells[70]. These results concluded that restoration of efficient ER α may serve as an important target for genistein where it will perform its anti-breast cancer function and genistein-related anticancer treatment will be an emerging treatment option in the future.

4. Epidemiological evidences for soy isoflavones

It has been observed in most epidemiological and experimental studies that there is an inverse correlation between soy isoflavones intake and breast cancer risk. Large numbers of studies have been conducted in Asian population due to the different food preferences especially high consumption of dietary isoflavones in their diet[71,72].

Different studies point out that dietary isoflavones can improve and predict breast cancer prognosis in breast cancer patients when they are part of their regular diet. According to one large cohort study that was conducted on around 11 000 breast cancer patients, it indicated that soy intake may be used as a promising therapeutic option mainly for ER (–) breast cancer in postmenopausal women[73]. Zhang *et al*. revealed that high genistein intake mimicking consumption patterns in Asian population increased the response of breast cells tumors to tamoxifen treatment, and this effect was associated with prosurvival autophagy genes, reduced the activity of unfolded protein response and increased anticancer immunity response to combat this frightful disease[74].

In human studies, the application of dietary isoflavones for breast cancer patients has been debatable up to some extent among the scientific community because of their double actions, namely, estrogenic and antiestrogenic actions. However, Chi *et al*. performed a meta-analysis study on large numbers of individuals and found that soy isoflavones intake reduced the occurrence and mortality of breast cancer[73]. Similarly, Guha showed reduced breast cancer recurrence while increasing daidzein isoflavone intake in a prospective cohort study of postmenopausal women where the treatment method was the application of tamoxifen at some point[75]. Large numbers of breast cancer cases arise in postmenopausal women while targeting breast cancer with these isoflavones[76]. It has also been examined that these isoflavones induce side effects that are similar to the sign and symptoms of menopause in breast cancer patients[77]. Lu *et al*. observed that females who regularly took soy isoflavones in their diet for around one complete month continuously had lower plasma level of estradiol and observed 3 days' average increase in their normal monthly cycle[78]. The reduced circulating estradiol level may be responsible, in part, for genistein's ability to reduce breast

cancer risk[78]. Shu *et al*. noticed that females who took soy products after being diagnosed with breast cancer had significantly decreased recurrence compared with those females who took very less amount or no soy[79]. It is still not clear that how the intake of soy isoflavones specifically affect breast cancer recurrence. However, we can predict that these results rely on expression of different genes triggered by these soy isoflavones. For instance, Maskarinec *et al*. found that females who took dietary isoflavones in high amount at young ages had lower level of PCNA and HER2/neu staining in malignant breast tissue[80].

5. Conclusions and future perspectives

After a detailed literature survey, it can be concluded that dietary isoflavones are performing double actions: estrogenic and anti-estrogenic functions on breast cancer cells. Isoflavones have also shown opposing effects, such as cell proliferation and apoptosis in breast cancer tissues in both types of studies: *in vitro* and *in vivo*. This might be due to the reason that cells exposed to soy isoflavones in culture respond in different ways than their cells in model animals in its natural environment. Additionally, some signaling cascades that are present to cells in culture are actually different compared with those present in an animal model.

As we talked earlier in this article, that dietary isoflavones consumption at young age boost up immunity against breast cancer[30,55]. Recently, several research studies support this fact that soy isoflavones induce epigenetic properties, reduce DNA methylation[81] and modulate histone acetylation[82]. In addition, genistein has synergistic effect on chemotherapeutic agents such as doxorubicin[83] and trastuzumab[84].

In addition, some novel high-throughput approaches such as transcriptomics[84], cell culture proteomics[85], and metabolomics[86] are rapidly gaining more and more interest as compared to conventional *in vitro* assays. Advances in DNA microarrays, NMR and LC-MS techniques, 2-D electrophoresis and labeling methods will provide deeper understanding of these dietary isoflavones actions on nuclear and cellular level in breast cancer cells.

In parallel, a great deal of attention is given in order to improve the therapeutic potential of these isoflavones. As genistein does not possess more suitable physiochemical properties to drug formulation, therefore, new strategy has been formulated in order to design genistein-loaded liposomes[87] and genistein-loaded biodegradable nanoparticles[88] are highly soluble. Moreover, semi-synthetic derivatives of genistein are designed which are structurally modified by coordination with copper (Ⅱ)[89], 21-hydroxylation[90] or conjugation with polysaccharides[91,92] and still scientists are conducting researches to develop more synthetic derivitives which are highly potent in its anti-tumor effect in comparison to its parent genistein.

Novel strategies for breast cancer treatment are in progress to develop multi-target drugs so as to stop the activation of these molecular pathways that lead to drug resistance. As we know better that soy isoflavones are pleiotropic in their functions, target many signaling cascades and are promising naturopathic agents for the treatment of breast cancer[93].

Everything considered, soy isoflavones still need extensive research and attention of the scientific community to give deeper understanding of its chemopreventive properties at nuclear and cellular levels. Once the molecular mechanisms at nuclear and cellular level of these dietary isoflavones are addressed, *in vivo* experimentations must be performed to authenticate the preclinical results. Together, these research studies will provide deeper insights regarding the chemopreventive and chemotherapeutic role of these isoflavones in future for the treatment of breast cancer.

Conflict of interest statement

The authors declare no conflict of interest.

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