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Consumption of soybean, soy foods, soy isoflavones and breast cancer incidence: Differences between Chinese women and women in Western countries and possible mechanisms

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Abstract

Breast cancer is one of the most lethal diseases world-wide. However, there is a large difference in breast cancer incidence among Caucasian, Hispanic, African and Asian (e.g. Chinese) women with Caucasian women being the highest and Asian women being the lowest. It has been suggested that the dietary factors may account for approximately 50% of the breast cancer. One of such dietary components which are typical to Asian but not Caucasian diet is soy foods. A number of epidemiological studies have suggested that increasing soy consumption could be related to the decreased risk of occurrence and/or mortality of breast cancer. In this review, we first described briefly different types of soy products and their nutritional functions and consumption. Then, we described briefly soybean isoflavones, i.e. genistein (GEN), daidzein, glycitein, and presented several lines of evidence to demonstrate the possible association of soy flavone food consumption with incidence and prognosis of breast cancer; finally, we summarized several possible molecular mechanisms, including the effects of GEN as an agonist of ERβ, epigenetic and genome-wide effects, activation of peroxisome proliferator-activated receptors, induction of apoptosis and stimulation of autophagy, involved in the chemo-preventive effects of GEN on breast cancer.

Keywords: Breast cancer incidence; Genistein; Soybean; Soy foods; Soy isoflavones

1. Introduction

Breast cancer is one of the most lethal diseases in women in Western countries [1]. While the incidence of breast cancer in Caucasian women is higher than that in Hispanic and Asian women, the incidence of breast cancer has been increasing in China [1]. The precise etiological factors for breast cancers are still not clear. It has been indicated that different dietary factors partially account for the different incidence of breast cancer among Caucasian, Hispanic and Asian women [2,3]. In terms of the dietary factors, there is a large difference in consumption of soybean products between Asian women and women in Western countries. A number of epidemiological studies have suggested that increasing soy consumption appears to be related to the decreased risk of recurrence and/or mortality. In this review, we first described briefly types of soy products and their nutritional functions, consumption and production. Then, we presented several lines of evidence demonstrating that the association of soy food consumption with incidence and prognosis of breast cancer; finally, we summarized several possible molecular mechanisms involved in the chemo-preventive effects of GEN on breast cancer.

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2. Soybeans, types of soy food products and their nutritional functions and consumption

2.1. Nutritional value and health benefits of soybean

Soybean (*Glycine max* (L.) Merr.) (Fig. 1), also called "Shu" in ancient Chinese, is one of the five main plant foods in China along with rice, wheat, barley and mille. Soybean is orginated in

Fig. 1. The soybean pods, soybean seeds and Tufu.

China and has been cultivated for about 5000 years [4,5]. Soybean was first introduced to Southeast Asia, then to Europe in 18th century [4] and to America in 19th century [6]. Since 1940s, soybean has become one of the most important economic crops in America. Currently, United States is the largest soybean producer of the world, making up more than 35% of the worldwide production in 2011/2012 season [7]. Soybean has been widely cultivated in the world with a world-wide annual planting area of 102.77 million hm^2 and a harvest of 239.36 t in the season of 2011/2012 [7], generating an economic impact of $114 billion to farmers around the world.

United States, Brazil, Argentina and China are the current leaders of soybean production in the world, with a combined harvest of 205.27 t in the season of 2011/2012, making up 86% of the worldwide production [7]. In the past 11 years, worldwide production of soybeans has been increasing [8].

The biggest commercial interest in soy is the oil and protein. Soybean compositions vary depending on variety, location, climate and farming practices [8]. Dry mature raw soybean typically contains 8.5% moisture, 36.5% protein, 19.9% lipids, and 9.3% dietary fiber, according to USDA nutritional database [9]. Protein and lipid combined make up more than 60% of soybean on dry weight basis.

Before the recent surge in interests in protein of soybean, soybean oil was the main purpose of commercial production of soybean. According to USDA report, soybean oil is the second largest vegetable oil produced in the world with a total worldwide production of 42.92 million t, second only to palm oil's 55.29 million t in the year 2012/2013. Soybean oil contains 15.6% total saturated fatty acids, 22.8% total monounsaturated fatty acids, and 57.7% total polyunsaturated fatty acids [9]. Fat has been treated as an important issue in human diet for a long time and many scientific studies have focused on the impact of consuming different types of fat to human health. In the most recent release of the Dietary Guidelines for Americans, USDA expert panel recommends consuming less than 10% of calories from saturated fat and replacing them with monounsaturated and/or polyunsaturated fat that is associated with lowering risk of cardiovascular disease [10]. Soybean oil contains a very high level of unsaturated fatty acid and a significant amount of omega-3 fatty acids [11], which is considered as part of the healthy fat group. Alpha-linolenic acid (an omega-3 fatty acid) in soybean oil is an essential fatty acid for human nutrition, which means it cannot be synthesized by humans. Regular consumption of foods rich in omega-3 fatty acids can provide many health benefits, including reduced cardiac deaths.

As more about soybean understood, the focus has shifted to its other components in the past few decades, especially the protein. Protein provides amino acids to human diets. Proteins from different sources have different amino acid compositions, which will affect its nutritional value to human diets especially the essential amino acids. There are various methods to evaluate nutritional quality of food proteins. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) method is currently the most accepted method for such purpose, replacing the Protein Efficiency Ratio (PER) method in 1989. Soy protein's PDCAAS score (1.0) is ranked the highest among vegetable proteins, and is equal to that of milk proteins (casein, whey protein) and egg protein, indicating that soy protein provides complete amino acids to human nutrition [12]. In comparison, wheat gluten, another popular plant protein commonly found in vegetarian diet, only has a PDCAAS score of 0.25.

Recently, FAO recommended a new method of evaluating protein quality – Digestible Indispensable Amino Acid Score (DIAAS), which is said to correct some of the limitations of PDCAAS method [13]. This no doubt will change the way people evaluate various proteins for their nutritional value. However, there is not abundance of data available to compare various proteins' nutritional value using this new method due to the fact that this recommendation was just recently released by FAO.

Unlike other proteins, soy protein's health benefits reach far beyond just providing amino acids. There have been numerous studies on this subject in the past few decades. For instance, Anderson et al. [14] summarized 37 primary studies, and concluded that consumption of soy protein rather than animal protein significantly decreased serum concentrations of total cholesterol, LDL cholesterol and triglycerides. Its mechanism was under extensive study. Crouse III et al. [15] established the direct link of naturally occurring isoflavones in soy proteins and the lowering of total and LDL cholesterol. However, another study seemed to indicate that isoflavones did not play a key role in soy protein's cholesterol lowering effect [16].

On October 26, 1999, based on all the scientific evidences, FDA issued a final ruling on health claim of soy protein petitioned by Protein Technologies International. The ruling states that diet low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease. In the ruling, FAD proposed that the soy food should contain 6.25 g soy protein per serving in order to qualify for this health claim [17]. This is the most significant event for the soybean growing and processing industry for the past few decades.

Since FDA's approval of soy health claim, many studies have further demonstrated the link between soy product consumption and lowering the risk of CHD. In 2003, Hermansen et al. [18] reviewed 50 research studies and confirmed the positive relation between soy consumption and improving cholesterol profile. In 2011, Anderson et al. reviewed a total of 43 studies from 1996 and 2008 and concluded that consuming a median of 30 g soy protein per day significantly improve the lipoprotein risk factor for CHD. However, soy protein health benefit is not without controversies. In 2006, American Heart Association Science Advisory panel reviewed 22 studies found that isolated soy protein with isoflavones has minimal effect in lowering LDL. Although the finding does not agree with other studies, the panel still recommends that soy products should be beneficial to cardiovascular and overall health because of their high content of polyunsaturated fats, fiber, vitamins and minerals and low content of saturated fat [19].

FDA's approval of soy health claim serves as fuel for soy products market growth in U.S. market and dramatically changes the status of soy in mainstream American's diet. Between 2000 and 2007, more than 2700 new soy-based food products were introduced in U.S. market, and the soy market grew 4.5 times to 4.5 billion between 1996 and 2009 [20]. This market grew to 5.2 billion in 2011 [21]. According to information presented on the website of Soy foods Association of North America, energy bars and soymilk are the two largest categories with soy-based energy bars overtaking soymilk's No. 1 position in 2011. Energy bars market also represents the largest growth from 2010 to 2011 with a 14.7% increase in total sales [21].

With extensive conventional news media's coverage and information flow in the modern media such as Internet, Facebook, and Twitter, soy's healthy image has been established among many consumers young and old. United Soybean Board has been monitoring the consumers' attitude to soy for many years. In 1998, 67% of consumers considered soy products as healthy. This percentage increased to over 80% in 2005. It was slightly down since reaching the peak of 85% in 2007 and 2008 to 81% in 2011 [22] (Fig. 2).

With better understanding of soy protein's health benefit and consumers' increasing acceptance, soy product use has reached a much wider market. In 2000, USDA approved the use of some soy products in school lunch program [23]. This set a milestone in soy industry because it was the first time soy products could be used in USDA school lunch program as 100% of the serving instead of as an additive. Study in middle school of Maryland, USA showed that students accept soy-based products just as well as other popular school lunch items [24] In February of 2012, USDA further approved tofu and soy yogurts as a credit for meat/meat alternative component in school meal planning [25]. This policy became effective on July 1, 2012.

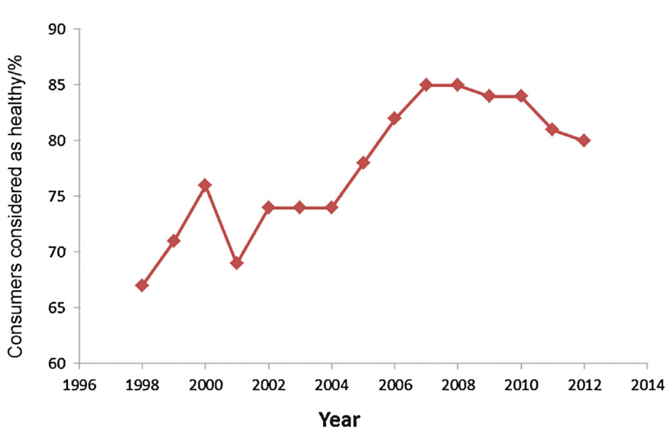

Fig. 2. Survey results of consumers' attitudes to soy [22].

The explosion of research on subject of soy and human health produced large amount of information, giving a much deeper understanding into soy's health benefits from molecular level. Many subjects have been extensively studied. Since 1994, the International Symposium on the Role of Soy in Health Promotion and Chronic Disease Prevention and Treatment was organized on a mostly bi-annual basis with the 9th symposium held in Washington, DC, October 2010. Scientists from around the world exchanged their scientific findings and knowledge in various health issues impacted by soy consumption, including cholesterol, heart disease, breast cancers, prostate cancers, bone health, menopausal symptom, weight loss, renal function, and cognitive function, to name a few [26,27]. Based on all the findings, soy products can play an important and positive role in improving our health if being incorporated into our diet although there are still much to be understood, especially the mechanisms.

2.2. Selected products derived from soybean

Edamame – Edamame is immature soybeans sold in fresh or frozen forms. As a traditional ingredient in Chinese and Japanese cuisine, Edamame can be found in many non-Asian restaurants in recent years, especially as an ingredient in salad. Frozen Edmame can be easily found in many mainstream grocery stores.

Soybean sprouts – Commonly found in Asian cuisine, especially Chinese, soybean sprouts are the germinating seeds of soybean under controlled conditions. Compared to mature soybeans, soybean sprouts contains substantial amount of good proteins and much higher amount of various vitamins [9].

Soynuts – In recent years, soynuts has been gaining popularity as a snack in Western world. Soynuts are made from soaked soybeans that have been fried, baked or roasted. Various flavors are then applied topically to soynuts to make the finish products.

Soybean flours – Soybean flours, or soy flours, are made from roasted soybean that have been ground into fine flour. Soy flours can be found as full fat or defatted versions. It is widely used in industry as an ingredient to boost the protein contents of many food items such as bakery and pasta.

Soybean oil – Soybean oil is one of the most important vegetable oil in the world. In 2012/2013, soybean oil makes up 27% of the world vegetable oil production, second to palm oil (35%) [28]. The recent surge of biofuel industry further expands the demands of soybean oil [29].

Soybean meals – Soybean meals is the ground soybean cake from which soybean oil has been extracted. The high protein content (>40%) makes it an important protein source for feed industry [30,31].

Soymilk – Soymilk is said to be invented in China by the legendary An Liu about 2000 years ago [32] with the first written record in AD 82. It is the aqueous extract of soy protein. Soymilk contains similar amount of protein and fat as cow's milk, and it is free of lactose and cholesterol. Therefore, it is considered as a healthy beverage and a popular alternative to dairy milk for population that is lactose-intolerant.

Tofu – Like soymilk, tofu is also invented in China. It is a curd made by coagulating soy protein by mineral salts or acid. It is much similar to the fresh cottage cheese in Western world which was made by coagulating cow's milk. In tofu making, a coagulant (calcium, magnesium salt, or glucono delta-lactone) is used to precipitate the soy protein from soymilk to form a jello-like curd. All tofu products involve the coagulation step. Depending on the downstream process, this curd can be converted into different types of products that can be found from today's supermarket. The jello-like curd can be further pressed to make tofu with various hardness and moisture levels, yielding soft, firm, extra firm, and super firm tofu. Various flavors can be added to tofu to create even more varieties. Historically, tofu is a major source of protein in the Chinese diet in which meat supply was not in abundance until recent years. Calcium-coagulated tofu also serves as an important source of calcium, an essential macro mineral, which is deficient in typical Chinese diet.

Unfortunately, tofu products vary greatly in their compositions even within the same variety because there is no established standard of identity. Different manufacturers choose their own standard for the same type of products, resulting in lots of confusion to consumers. Silken, soft, firm and extra firm tofu produced by several major tofu producers in U.S. contain varying amounts of proteins [33].

Consumers who are looking for tofu as a protein source will face a great challenge of differentiating products from different brands not only from its appearance, texture, flavor, but also need to pay attention to its protein content. Standardizing various varieties of tofu products based on composition will certainly help to further promote the products to consumers.

Okara – Okara is the residual solids from soymilk extraction. Okara typically contains about 80% moisture, 3.2% protein, and 1.7% fat on as-is basis [9]. Okara from different manufacturers can vary greatly depending on the manufacturing techniques. Although okara can be found as a cooking ingredient in China, Japan and Korean cuisine, its most popular use is still as feed materials for livestock. However, as people understand more about human needs for fiber as a nutrient in human nutrition, interest in application of okara as food ingredients has been increased in recent years. A simple Google search of "Okara Recipes" yields over 100,000 listing, indicating the rising interests in this unique ingredient derived from soybean. In Japan, okara is used as an ingredient to manufacture extruded okara snacks.

Natto – Natto is a traditional Japanese soy foods made by fermenting soybean with strains of *Bacillus subtillis natto* [33]. Like some very strong cheeses, Natto's smell, flavor, and its slimy texture could be challenging for some people to accept. Nevertheless, it has become much more popular in recent years among U.S. consumers due to its perceived health benefits.

Tempeh – Tempeh is a fermented soybean product originated in Indonesia. It is made by fermenting dehulled and partially cooked soybeans with *Rhizopus* mold [34]. The extensive network formed by mycelia binds the soybean together to form a block, making it like a fermented soybean loaf. Besides being a high protein food, co-fermentation by some bacteria in Indonesian Tempeh also produces VB_{12}, an essential vitamin that is typically deficient in pure vegetarian diet [35].

Miso – Miso is a popular Japanese food made by fermenting rice and soybean with a mixture of molds, yeast and bacteria. Traditional miso manufacturing process starts with fermenting cooked rice with Koji spores (spores from *Aspergillus Oryzae*), followed by second fermentation of the fermented rice and cooked soybean mix by molds, yeasts and bacteria, yielding a paste material with complex flavor [36]. In U.S., miso is most commonly used in miso soup and as seasoning for flavor.

Soy sauce – Soy sauce is the most common seasoning in China and has a recorded history as early as 160 AD [37]. Soy sauce is called Jiang You in China, Shoyu or Tamari Shoyu in Japan, Ketjap or Kecap in Indonesia, Kicap in Malaysian, and Kanjang in Korea [37]. Similar to miso, traditional soy sauce is manufactured by fermenting a mix of wheat and soybean by various microorganisms including *Aspergillus*, lactic acid bacteria and yeasts [38] creating a very complex flavor and aroma through chemical and biochemical reactions during the manufacturing and processing steps. Various brands of soy sauce are very different in flavor and aroma due to its variations in raw material formulation, starter cultures, and fermentation process. In recent years, healthy eating trend also promotes the popularity of low salt soy sauce. In modern manufacturing technique, soy sauce is also made by hydrolysis process, yielding a product that is considered by many as inferior.

Soy protein isolate – Soy protein isolate is soy protein isolated from soybean ingredient such as soy meals. It contains very high level of protein (>90%), and it is nearly carbohydrate and fat-free [39]. Soy protein isolate is widely used in food industry in protein drink dry mixes, infant formula, soups, liquid nutritional meals, soymilk, frozen desserts, dressings, bakery, breakfast cereals, pet foods, pasta, cheese alternatives, snack foods, and protein supplement products.

Texturized meat alternatives – As food processing technology advances, soy protein, either alone or in combination with other vegetable proteins, can be processed into various texturized meat substitute products. This type of product, either in dry, refrigerated or frozen form, is typically similar to and color matching the corresponding meat. They can be in the form of plain ingredients or formulated products or in the form of formulated products such as vegetarian meat ball and vegetarian sausages. This substantially improves the appeals of the products to mainstream consumers, and expands the choices of protein to the vegetarian and healthy eaters.

Cheese and dairy alternatives – Soy cheese is a cheese analog made with soy derived ingredients or tofu. These

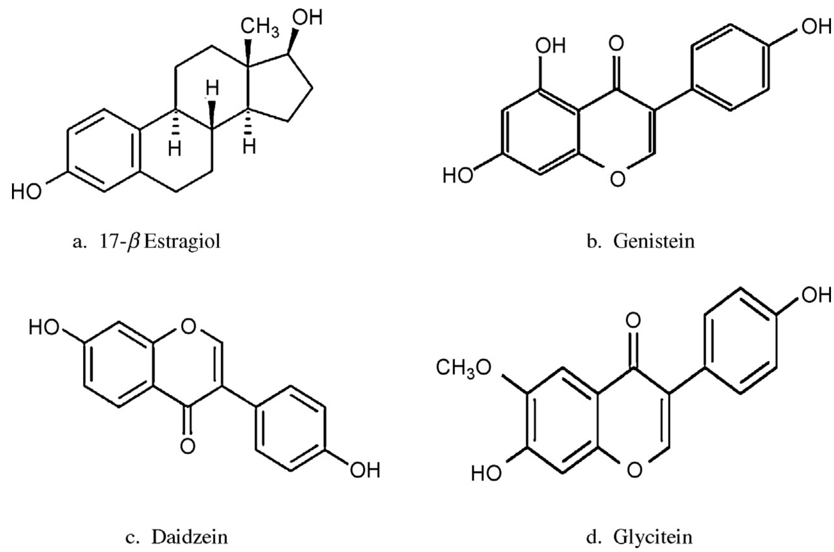

Fig. 3. Chemical structures of genistein (GEN), daidzein, and glycitein and 17-β estradiol.

products, such as various varieties of cheese block, slices, and cream cheese, can be easily found from local health food stores or the health food section of local grocery stores. Similar to the dairy counterparts, soy cheese comes in various flavors. However, unlike the dairy versions, the current versions of common soy cheese and dairy alternatives are not fermented products [40]. It is much similar to the processed cheese or processed cheese foods in dairy market.

Soy yogurt – Soy yogurt is fermented soy based product using soymilk as substrate. Soy yogurt production process is similar to traditional yogurt manufacturing in which starter cultures are used to ferment the protein-rich base substrate, yielding a product that contains healthy soy protein and beneficial probiotics cultures.

Nondairy desserts – Soy based ice cream, soy whipped cream topping, soy-containing frozen desserts and bakery items such as soy cheese cake have been in existence for many years, although its popularity has not yet reached mainstream.

Nutritional supplements – Various nutritional or nutraceutical supplement products have been produced from soy bean processing. The most popular ones are Vitamin-E, Lecithin and Isoflavone, which can be found in most pharmacies and health supplement stores in U.S.

3. Soybean isoflavones: genistein, daidzein, glycitein, and formononetin

While proteins, soy bean oil and carbohydrates are the main components of soy, which are healthy components, soy foods described above also contain varying concentrations of phytoestrogens (PE) called isoflavones, which are responsible for many of health benefits including their effects in breast cells.

3.1. Structures and biosynthesis of genistein (GEN), daidzein, and glycitein

The phytoestrogens (PE) with estrogen-like structures can be classified into four distinct types: isoflavones, coumestans, lignans and prenylflavonoids. Among PEs, isoflavones are present in high concentrations in soy and soy products [41,42]. The chemical structures of several isoflavones, genistein, daidzein, glycitein, and formononetin, are shown in Fig. 3. Isoflavones are usually present as glycoside conjugates in foods, such as genistin, diadzin, and glycetin, or their aglycone forms such as genistein, diadzein, and glycetein. While isoflavones occur in many types of legumes, soybean contains the highest concentration of isoflavones. Soybean contained a combined amount of 61.7 mg of diadzein (37.6 mg) and genistern (24.1 mg) per kg of day weight [43]. Diadzin/diadzein, genistin/genestein and glycetin/glycitein typically account for 40%, 50% and 10% of total isoflavones, respectively [44]. The relative amounts of diadzin/diadzein, genistin/genestein and glycetin/glycitein vary among different cultivars and geographical locations [45–48]. For example, it has been reported that isoflavone content in commercial soybean foods was in the ranges of 1176–3309 μg/g across years [45] and isoflavone content of soybeans in Asia varied from 699.7–2581.6 μg/g with cropping year [46], in Canada varied from 360–2241 μg/g [47] and in Europe (Romania) varied from 712 to 1228 mg/g, respectively [48]. Mature soybean seeds from Korea and United States contained the highest amounts of total isoflavone content of (178.81 ± 41.17) mg/100 g and (159.98 ± 43.58) mg/100 g, respectively (Table 1) [49]. The detailed amounts of diadzin, genistin, and glycetin in a wide variety of soybean organs and soy foods (e.g. soy protein concentrates soymilk and fermented products such as miso, temph, sufu and tofu) are listed in Table 1 [49], among which, the mature soybean seeds contain the highest amounts of diadzin, genistin, and glycetin and total amounts of these isoflavones. For soybean foods, a variety of soybean flours contain the highest amounts

Table 1
Summary of contents of isoflavones in soybean food products.[a]

NDB #	Food description	Daidzein	Genestein	Glycitein	Total	*N*	CC
99055	Soy cheese, Mozzarella	1.14 ± 0.64	2.60 ± 1.30	2.28 ± 1.06	6.02 ± 2.96	5	B
99056	Soy cheese, Parmesan	1.50 ± 0.00	0.80 ± 0.00	4.10 ± 0.00	6.40 ± 0.0	3	C
99471	Soy cheese, swiss	1.80	4.40	1.70	7.9	1	C
99042	Soy cheese, unspecified	5.79 ± 6.41	11.14 ± 11.31		25.72 ± 20.18	8	B
99043	Soy drink	2.75 ± 1.23	5.10 ± 1.80		7.85 ± 3.04	5	C
09945	Soy fiber	18.80 ± 1.41	21.68 ± 2.89	7.90 ± 0.00	44.43 ± 3.98	6	B
99080	Soy flour (textured)	67.69 ± 19.25	89.42 ± 26.96	20.02 ± 6.77	172.55 ± 50.01	35	B
116117	Soy flour, defatted	64.55 ± 20.20	89.42 ± 26.96	20.02 ± 6.77	172.55 ± 50.01	49 79 27 49	B
16115	Soy flour, full-fat, raw	72.92 ± 10.02	98.77 ± 20.21	16.12 ± .33	178.10 ± 37.06	60	B
16116	Soy flour, full-fat, roasted	89.46 ± 19.72	85.12 ± 21.35	16.40 ± 0.0	165.04 ± 51.03	6	C
16119	Soy meal, defatted	80.77 ± 9.17	114.71 ± 18.42	16.12 ± 1.02	209.58 ± 32.71	8	C
99049	Soy noodle, flat	0.09 ± 0.00	3.70 ± 0.00	3.90 ± 0.00	8.50 ± 0.00	3	C
99038	Soy paste	19.71 ± 5.32	17.79 ± 5.75	6.05 ± 3.01	38.24 ± 11.25	33	B
99060	Soy protein concentrated aqueous washed	38.25 ± 18.81	52.81 ± 8.35	4.94 ± 0.49	84.65 ± 25.75	11	B
16121	Soy protein concentrate produced by alcohol extraction	5.78 ± 3.83	5.26 ± 1.78	1.57 ± 0.00	11.49 ± 5.50	21	B
99660	Soy protein drink	27.98 ± 18.19	42.91 ± 25.44	10.76 ± 3.65	81.65 ± 46.04	8	B
16122	Soy protein isolate	30.81 ± 12.73	57.28 ± 14.17	8.54 ± 3.22	91.05 ± 26.00	49	B
99510	Soy yogurt	13.77 ± 12.39	16.59 ± 9.83	2.80 ± 4.12	33.17 ± 26.16	5	B
99072	Soy chips	26.71 ± 0.00	27.45 ± 0.00		54.16 ± 0.00	3	C
99034	Soybean, curd, fermented	12.18 ± 1.91	21.12 ± 1.15	2.30 ± 0.00	34.68 ± 3.89	5	C
99035	Soybeans, flakes, defatted	37.47 ± 24.37	91.22 ± 41.85	14.23 ± 0.00	131.53 ± 64.64	10	C
99036	Soybeans, flakes, full fat	21.75 ± 23.03	39.57 ± 43.82	1.12 ± 0.35	62.31 ± 67.07	9	B
99100	Soybeans, green, mature	61.70 ± 5.84	60.07 ± 12.13	7.07 ± 3.60	128.83 ± 18.66	15	B
99520	Soybeans, mature seeds, canned	26.15 ± 0.64	25.15 ± 12.87	6.1	52.82 ± 16.29	4	B
16109	Soybeans, mature seeds, cooked, boiled, without salt	30.76 ± 10.64	31.26 ± 8.83	3.75 ± 1.53	65.11 ± 10.57	28	B
16111	Soybeans, mature seeds, dry roasted (included soy nuts)	62.14 ± 28.04	75.78 ± 25.18	13.33 ± 8.69	148.50 ± 63.12	16	B
16108	Soybean, mature seeds, raw (all sources)	62.07 ± 20.01	80.99 ± 22.64	14.99 ± 7.45	153.53 ± 43.07	1000	B
99574	Soybeans, mature seeds, raw (Australia)	39.88 ± 16.98	65.64 ± 19.35	17.12 ± 4.10	120.84 ± 34.12	57	B
99030	Soybeans, mature seeds, raw (Brazil)	29.09 ± 12.70	67.57 ± 13.69	13.10 ± 3.58	99.82 ± 21.22	58	B
99488	Soybeans, mature seeds, raw (China)	53.33 ± 13.89	57.98 ± 5.60	11.71 ± 2.35	118.28 ± 21.20	22	B
99575	Soybeans, mature seeds, raw (Europe)	45.44 ± 10.54	39.78 ± 14.46	22.37 ± 8.19	103.56 ± 18.71	44	B
99092	Soybeans, mature seeds, raw (Japan)	45.95 ± 22.47	74.33 ± 23.56	9.01 ± 3.21	130.65 ± 41.17	49	B
99093	Soybeans, mature seeds, raw (Korea)	78.86 ± 19.72	89.32 ± 24.68	18.76 ± 7.13	178.81 ± 47.16	314	C
99040	Soybeans, mature seeds, raw (Taiwan)	22.77 ± 18.10	45.88 ± 16.78	13.24 ± 5.17	85.68 ± 33.42	22	B
99576	Soybeans, mature seeds, raw (United States)	61.33 ± 21.48	86.33 ± 20.30	13.33 ± 8.89	159.98 ± 43.58	399	B
16230	Soymilk (all flavors), nonfat, with added calcium, vitamins A and D	0.30	0.41	0.00	0.70	1	C
99568	Soymilk curd, dried	40.85 ± 1.42	43.45 ± 0.45		83.30 ± 0.89	6	B
99096	Soymilk skin or film (Foojook or yuba), cooked	17.81 ± 2.98	25.15 ± 5.31	2.69 ± 0.55	44.67 ± 8.12	11	B
99053	Soymilk skin or film (Foojook or yuba), raw	80.03 ± 21.28	101.40 ± 21.98	15.43 ± 2.99	196.05 ± 46.55	19	B
99014	Soymilk, iced	1.90 ± 0.98	2.81 ± 0.66		4.71 ± 1.64	6	C
99559	Soymilk, made from soy isolate (purchased in Australia)	2.80 ± 0.00	3.10 ± 0.00		5.90 ± 0.00	3	B
99572	Soymilk, original and vanilla, fortified or unfortified	4.84 ± 1.71	6.07 ± 0.47	0.93 ± 0.00	10.73 ± 3.99	155	B

Table 1 (*Continued*)

NDB #	Food description	Daidzein	Genestein	Glycitein	Total	*N*	CC
99497	Sufu	7.50 ± 2.77	5.46 ± 2.32	0.78 ± 0.46	13.75 ± 4.86	12	C
16114	Tempeh	22.66 ± 8.99	36.15 ± 17.64	3.82 ± 1.45	60.61 ± 27.44	28	B
99081	Tempeh burger	6.40 ± 0.00	19.60 ± 0.00	3.00 ± 0.00	29.00 ± 0.00	3	C
16174	Tempeh, cooked	13.12	21.14	1.39	35.64	2	C
99500	Tempeh, fried	32.90 ± 0.00	39.90 ± 0.00	NA	72.80 ± 0.00	3	B
43476	Tofu yogurt	5.70 ± 0.00	9.40 ± 0.00	1.20 ± 0.00	16.30 ± 0.00	3	C
99084	Tofu, Azumaya, extra firm, cooked (steamed)	8.0	12.75	1.95	22.70	1	B
99085	Tofu, Azumaya, firm, coked	12.8	16.15	2.40	31.35	2	B
16128	Tofu, dried frozen (Koyadfu)	29.59 ± 2.58	51.04 ± 5.41	3.44 ± 0.00	83.20 ± 9.56	4	C
99529	Tofu, firm, braised	7.28	8.22	1.28	16.79	4	B
99529	Tofu, firm, cooked	10.26 ± 2.48	10.83 ± 3.98	1.35 ± 0.32	22.05 ± 6.36	7	B
16126	Tofu, firm, prepared with calcium sulfate and magnesium chloride (nigan)	12.31 ± 4.72	16.10 ± 7.70	2.75 ± 1.28	30.41 ± 13.30	105	B
16129	Tofu, Fried	13.80 ± 2.70	18.43 ± 4.67	2.93 ± 1.13	34.78 ± 7.32	39	B
16162	Tofu, MORI_NU, silken, firm	12.42 ± 2.36	16.95 ± 2.49	2.40	29.97 ± 3.75	4	B
16130	Tofu, okara	3.62 ± 2.98	4.47 ± 2.86	1.30 ± 0.38	9.39 ± 6.20	7	B
99097	Tofu, presses (Tua kwa), raw	15.59 ± 2.16	16.01 ± 2.35	2.77 ± 0.85	33.91 ± 5.38	18	B
16427	Tofu, salted and fermented (fuyu)	8.56 ± 3.32	12.99 ± 4.19	1.98 ± 0.59	22.73 ± 7.33	10	B
16132	Tofu, salted and fermented (fuyu)	20.72 ± 9.09	23.83 ± 10.54	4.95 ± 0.04	48.51 ± 21.73	5	C
99495	Tofu, silken	9.15 ± 2.32	8.42 ± 1.50	0.92 ± 0.22	18.04 ± 3.74	25	B
99541	Tofu, smoked	7.50	5.60	NA	13.10	3	B
16127	Tofu, soft, prepared with calcium sulfate and magnesium chloride (nigan)	9.49 ± 2.19	11.91 ± 3.82	1.68 ± 0.53	22.61 ± 5.43	18	B
99086	Tofu, soft, Vitasoy-silken	8.59	20.65	NA	29.24	2	C
16147	Veggie burgers or soyburgers, unprepared	2.36 ± 1.04	5.01 ± 2.91	0.55 ± 0.45	6.39 ± 2.86	31	B

[a] Adapted and re-arranged from USDA Database for the Isoflavone Content of Selected Foods (U.S. Department of Agriculture: http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/isoflav/Isoflav) [49].

Units = mg/100 g; The data were presented as mean ± standard deviation, and the edible portion for mean. *N* = number of samples analyzed; CC = confidence code; NA = not available.

of diadzin, genistin, and glycetin and total amounts of these isoflavones.

In higher plants, isoflavones are biosynthesized via a branch of the general phenylpropanoid pathway, which begins from the amino acid phenylalanine naringenin, an intermediate of the pathway, is sequentially converted into the isoflavone genistein by isoflavone synthase and a dehydratase. Similarly, naringenin chalcone, another intermediate is converted to the isoflavone daidzein by sequential action of chalcone reductase, type II chalcone isomerase, and isoflavone synthase. Soybean uses isoflavones to stimulate soil-microbe rhizobium to form nitrogen-fixing root nodules [50].

3.2. Metabolism of isoflavones by intestine bacteria and difference in the metabolism rate of isoflavones between U.S. Caucasian population and Asian population

When taken in human intestine, soy isoflavones undergo extensive transformations during passage through human digestive tract, especially in the colon, where members of the complex commensal microbiota are capable of carrying out synergistically a broad range of metabolic transformations that affect the fate and the biological activity of phytochemicals. Diverse bacterial species present in the colon hydrolyze the glucose conjugated forms of isoflavones, releasing the corresponding aglycones, which may undergo further microbial conversions (especially reductions) giving rise to a wide spectrum of isoflavone-derived compounds.

Equol, one of the bio-transformed metabolites of isoflavones, is a more potent estrogenic metabolite, and possesses a greater affinity for estrogen receptors (ERs), unique antiandrogenic properties, and superior antioxidant activity [51]. Equol has an activity of approximately 0.2%–0.5% of estradiol [52,53]. Although this affinity is relatively low, equol is still capable of significantly competing with estradiol for ERs because of its large amount produced from soy products.

The end-products of microbial transformations are subjected to substantial individual variations, reflecting the impact of the colonic microbiota, since intestinal bacteria may greatly increase or compromise the biological activity of dietary isoflavones. The clinical effectiveness of soy isoflavones may be related to ability to biotransform soy isoflavones to equol [54]. There are large differences in the metabolism rates of GEN and daidzein between U.S. Caucasian population and Asian population. Only approximately 30%–50% of the general population is able to metabolize daidzein to equol. Only 25%–35% of the U.S. Caucasian population is capable of converting daidzein to equol whereas 40%–60% of Asian people in high soy consumption areas is capable of converting daidzein to equol. Hispanic or Latino women are also more likely to be equol producers.

Approximately 80%–90% of people harbor the bacteria required to produce *O*-desmethylangolensin (ODMA). The frequency of equol producers of vegetarians was found to be approximately 60%, similar to the reported frequency in Japanese adults consuming soy, and much higher than those for non-vegetarian adults (25%). One Japanese study found that consumption of dairy products was significantly higher in those who did not excrete equol than in those who did. It has been found that seaweed consumption can enhance intestinal production of equol. These observations could partially explain some of the breast cancer protective effects of Asian diets high in both seaweed and soy. Another U.S. study comparing Korean American and Caucasian American women found that the prevalence of equol-producers was higher (51% vs. 36%) whereas the prevalence of ODMA-producers was lower (84% vs. 92%) in the Korean Americans as compared to those of Caucasian American women [55].

The differences in equol production rate could partially explain the generally tepid effects found for soy consumption in studies of European and U.S. populations compared to Asian populations (see below). This high variability in equol production is presumably due to inter-individual differences in the composition of the intestinal microflora, which may play an important role in the mechanisms of action of isoflavones. It is possible that Chinese populations could have a genetic profile different from other populations that are relatively new to soy consumption because Chinese populations already have had more than 5000 years to develop their ability to digest and extract nutrients from soybeans.

4. The association of soy isoflavone food consumption with incidence and prognosis of breast cancer

4.1. Differences in breast cancer incidence between Asian women and women from Western developed countries in Europe

Breast cancer is one of the most lethal diseases in women with approximately 1.4 million women diagnosed with breast cancer and approximately 459,000 deaths in 2008 world-wide [56]. There are large differences in breast cancer incidence between Asian women and women in U.S. and the developed countries in Europe. For women in the U.S. and European countries, breast cancer death rates are higher than those for any other cancers, only second to lung cancer [56].

Compared to that in U.S. and European countries, breast cancer incidence in China and other Asian countries are much lower with the risk for an Asian woman having been recorded as nearly seven times lower in breast cancer risk than that for an American woman [57]. While breast cancer rates among Asian-Americans are lower than those of U.S. whites, cancer rates among Asian-Americans are considerably higher than those prevailing in women in Asian countries, suggesting that the increased risk factors are not only related to genetic factors but also related to environmental and dietary factors [58]. There are differences in breast cancer pathology and incidence rates across racial groups. Among women younger than 50 years, African Americans are not only at a greater risk for developing breast cancer but may also be more likely to present with more aggressive, steroid receptor negative, and higher-grade tumors [59–61].

Breast cancer is also the most common cancer diagnosed among Chinese women. A recent study has revealed that the crude incidence and cancer mortality of breast cancer in Chinese women are 47.64/100,000 and 10.41/100,000, respectively, and the cumulative incidence rate for women with ages ranging from 0 to 74 years is 3.44%, and that both crude and adjusted incidence rates for women in urban areas were much higher than those in rural areas [62]. A modeling analysis on the effects of reproductive and demographic changes on breast cancer incidence in China predicted that breast cancer incidence in China will be increased substantially from current rates, estimated at 10–60 cases/100,000 women, to more than 100 new cases/100,000 women who aged 55–69 years by 2021, i.e. there will be 2.5 million cases of breast cancer by 2021 among the 130 million Chinese women aged 35–49 years old in 2001 and that some changes in lifestyle that might reduce an impending epidemic of breast cancer by 27,000 cases [62,63,1]. The gradually increasing trend in breast cancer rates has also seen in other eastern Asian regions (e.g. Japan, Korea and Taiwan (China)) and southeastern Asian regions (e.g. Philippines, Singapore and Thailand) [64]. Increasing trends in developing counties are often considered as the result of the “westernization” of life styles [56], dietary habits and exposure to exogenous estrogen, toward a distribution closer in profile to that of women in industrialized countries.

The differences in genetics, endogenous hormone levels and hormone metabolism, growth patterns, diets and other environmental factors among Asian, U.S. Caucasian and African-American and Latino women may alter some of these relative risks quantitatively. Then, the key question is what are the key factors contributing to this discrepancy?

One of such factors could be the difference in endogenous levels of estrogens between Asian and Caucasian and African-American women. The racial differences in premenopausal endogenous hormones between African-American, Caucasian and Asian-American women were reported. For example, Goldin et al. [65] compared the plasma estrogen levels of premenopausal Caucasian woman living in Boston with those of Asian women recently immigrants from Southeast Asia to Hawaii and found that Caucasians had 30%–75% higher plasma estrone and estradiol levels than their age-matched cohorts in Hawaii, and that Caucasians had 3-fold higher plasma levels of estradiol. Pinheiro et al. [66] assayed estradiol, progesterone, prolactin, sex hormone binding globulin (SHBG), insulin-like growth factor-I (IGF-I), and IGFBP-3 in 111 African American and 111 Asian American women, matched to 111 Caucasian women on age, day of luteal phase, and day, time, and fasting status at blood collection to determine the association between race and hormone levels using robust linear regression methods. Their analysis revealed that African Americans had 18% higher levels of estradiol, 17% higher free estradiol. Compared with that of Caucasian women, Asian Americans had 22% higher calculated free estradiol. The hormone differences were found to be consistent with breast cancer risk between Caucasians and African Americans but were inconsistent with breast cancer risk between Asian Americans and Caucasians [66]. It has been known that prolonged exposure to excess estrogens could be an important risk factor for the development of breast cancer [67]. Thus, the difference in endogenous levels of estrogens among Asian-, Caucasian- and African-women may partially explain the differences in breast cancer incidence among different racial women.

Another important factor that may account for the discrepancy in breast cancer incidence among different racial women could be the differences in their daily dietaries. There are differences in dietaries particularly in consumption of soybean products between women in Western countries and women in Asian countries. Approximately 50% of all newly diagnosed breast cancers could be attributed to diets. Thus, identification of the dietary factors differentially consumed among populations with increased breast cancer risk (e.g. Caucasians) compared to those with low risk (e.g. Asians) would offer new etiologic insights and opportunities for prevention and reduction of breast cancers. One such dietary component which is typical to the Asian but not the Caucasian diet is the consumption of soy foods.

This is evident by the fact that the breast cancer incidence for Asian women who immigrated to U.S. was higher than that of women in Asian countries. It is presumed that migration to the U.S. brings about a change in endocrine function among Asian women and that the high intake of soy foods in Asia and its reduced intake among Asian-Americans partly explain the increase of breast cancer rates in Asian-Americans. For example, Wu et al. [58] conducted a population-based case–control study of breast cancer among Chinese-, Japanese-, and Filipino-American women in Los Angeles County, MSA, San Francisco and Oakland in California and Oahu in Hawaii. They found that intake of a soybean product, tofu, was more than two times as high among Asian-American women born in Asia compared to those born in the U.S. Among migrants, intake of tofu decreased with years of residence in the U.S. Breast cancer risk decreased with increasing frequency of intake of tofu after adjustment for age, study area, ethnicity, and migration history; the adjusted OR associated with each additional serving per week was 0.85 (95% CI = 0.74–0.99). The protective effect of high tofu intake was observed in pre- and post-menopausal women. The question is whether soy, and more specifically soy isoflavones (e.g. GEN), is a dietary component that may help to explain the dramatic disparity in breast cancer risk among these populations [58].

4.2. The association of isoflavone-containing soy food consumption with breast cancer prognosis

A number of studies have been conducted to investigate the association between the soy food consumption and breast cancer incidence/prognosis (see summary in Table 2). Qin et al. [68] performed a meta-analysis on 21 independent studies including 14 case–control studies and 7 cohort studies from 1966 to 2006 and examined the association between soy food consumption (i.e. isoflavone intake) and breast cancer risk. They used the random-effects model to estimate the pooled relative risk (RR). The pooled RR of breast cancer for soy food intake was 0.75 with a 95% CI of 0.59–0.95. This study showed that tofu and miso, two main types of soy foods in Japan and China, had clear protective effects and that isoflavone intake resulted in a 20% decrease in breast cancer risk. Additionally, when the studies published in both Japanese and Chinese were taken into account, an inverse association between soyfood tofu and breast cancer risk was relatively stronger. This meta-analysis supported the hypothesis that soy food intake could be associated with a decreased breast cancer risk due to the isoflavones.

A number of subsequent epidemiological studies have been conducted with more comprehensive information about the soy food, and more accurate assessment of the isoflavones. For example, Guha et al. [69] examined the role of soy isoflavone intake and the incidence of breast cancer risk by estrogen receptor (ER) status, menopausal status, and tamoxifen (TAM) therapy. A cohort of 1954 women breast cancer survivors, diagnosed during 1997–2000, was prospectively followed for 6.31 years and 282 breast cancer recurrences were ascertained. This study revealed: 1) a trend for a reduced risk of cancer recurrence with increasing quintiles of daidzein and glycetin intake compared to no intake among postmenopausal women; 2) among postmenopausal women treated with TAM, there was an approximately 60% reduction in breast cancer recurrence as compared to the highest to the lowest daidzein intakes and 3) soy isoflavones consumed at levels comparable to those in Asian populations may reduce the risk of cancer recurrence in women receiving TAM therapy and moreover, appears not to interfere with TAM efficacy.

Several epidemiological studies [70–76] evaluated the association between soy food intake and breast cancer risk of women in different regions in mainland China. For example, Shu et al. [70] investigated a cohort with 5033 women aged from 20 to 75 years old who were diagnosed between 2002 and 2006 recruited and followed up through 2009. This study revealed: 1) soy food intake was inversely associated with mortality and recurrence and 2) this trend was independent of ER status or TAM usage. Those in the highest quartile of soy protein intake not taking TAM had less recurrence than TAM users consuming the least amount of soy. In another study, Kang et al. [71] recruited 524 patients in northwest region in China who underwent surgery for breast cancer between August 2002 and July 2003 and received adjuvant endocrine therapy. Their results indicated that high dietary intake of soy isoflavones was associated with lower risk of recurrence among post-menopausal patients with breast cancer positive for ER and progesterone receptor (PR) and those who were receiving anastrozole as endocrine therapy. Zhang et al. [72] consecutively recruited 438 patients with primary breast cancer and compared them with 438 health women in Guangdong province (South China) from June 2007 to August 2008. They observed a statistically significant inverse association of soy isoflavone intake with breast cancer risk, which was more evident among premenopausal women. In consistent with this observation, a case–control study by Zhu et al. [73] with a total of 183 patients with breast cancer and 192 controls recruited from 2008 to 2011 in south China also indicated an association of the highest relative to lowest soy isoflavone intake with a 58% decrease risk of breast cancer. Higher

Table 2
The association of isoflavone-containing soy food consumption with breast cancer recurrence/prognosis.

Authors/reference	Year	Type of study/number of patients Country/regions	Major findings/conclusions	Statistical parameters/outcomes
Qin et al. [68]	2006	Mega-analysis (14 case–control studies and 7 cohort studies) International	A 20% reduction in breast cancer risk. Soyfood intake may be associated with decreased risk of breast cancer	RR = 0.81; 95% CI: 0.67–0.99
Guha et al. [69]	2009	Prospective study Cohort/1954	Soy isoflavones consumed at levels comparable to those in Asian populations may reduce the risk of cancer recurrence in women receiving tamoxifen therapy and not to interfere with tamoxifen efficacy	HR: 0.48; 95% CI: 0.21–0.79, $p = 0.008$
Shu et al. [70]	2009	Population-based cohort study/5042 female breast cancer survivors in Shanghai/China	Soy food intake, as measured by either soy protein or soy isoflavone intake, was inversely associated with mortality and recurrence	HR: 0.71; 95% CI: 0.54–0.94 for total mortality 95% CI: 0.54–0.87 for recurrence
Kang et al. [71]	2010	Follow up study with 524 patients underwent surgery for breast cancer in Northeast China	High dietary intake of soy isoflavones was associated with lower risk of recurrence among post-menopausal patients with breast cancer positive for ER and PR and those who were receiving anastrozole as endocrine therapy	HR: 1.05, 95% CI: 0.78–1.71; p for trend: 0.87 for overall death rate HR = 0.67, 95% CI 0.54–0.85, p for trend = 0.02 for recurrence
Zhang et al. [72]	2010	A hospital-based case–control study/438 Chinese women with breast cancer residing in Guangdong province (South China)	A statistically significant inverse association between soy isoflavone and soy protein intake with breast cancer risk	95% CI: 0.54 (0.34–0.84) for soy isoflavone 95% CI: 0.62 (0.40–0.96) for soy protein
Zhu et al. [73]	2011	A case–control study/183 women with breast cancer in South China	The highest relative to lowest soy isoflavone intake was associated with a 58% decrease risk of breast cancer; a high intake of soy food was inversely associated with breast cancer risk, the effect depending to some extent on the hormone receptor status (ER^+/PR^+)	OR = 0.57, 95% CI: 0.29–0.83 OR: 0.50, 95% CI: 0.38–0.95 for highest intake of soy isoflavone and soy protein
Kang et al. [74]	2012	A prospective study with 256 patients with breast cancer in Inner Mongolia (North China)	Soy intake is associated with a significant reduced death risk of breast cancer in Chinese population	OR: 0.25, 95% CI: 0.09–0.54 for highest soy isoflavone OR: 0.38, 95% CI: 0.17–0.86 for the higher consumption of soy protein
Zhang et al. [75]	2012	A prospective study with breast cancer patients in Inner Mongolia (North China)	An average intake of soy isoflavone above 17.3 mg/day reduced the mortality of breast cancer by about 38%–36%	HR: 0.71; 95% CI: (0.52–0.98) for high soy protein intake HR: 0.59, 95% CI: 0.40–0.93 for high intake of soy isoflavone
Dong et al. [76]	2011	A meta-analysis of prospective studies/International Populations	Soy isoflavones intake is associated with a significant reduced risk of breast cancer incidence in Asian populations, but not in Western populations	RR: 0.89, 95% CI: 0.79–0.99 for isoflavone consumption RR: 0.76, 95% CI: 0.65–0.86 for Asian populations RR: 0.97, 95% CI: 0.87–1.06 for Western populations
Nechuta et al. [77]	2012	A prospective study with 5514 breast cancer survivors with a diagnosis of invasive breast cancer from 2 US cohorts and 1 Chinese cohort.	Consumption of ≥10 mg isoflavones/day was associated with a nonsignificant reduced risk of all-cause and breast cancer-specific and a statistically significant reduced risk of recurrence Postdiagnosis soy food consumption of ≥10 mg isoflavones/day was associated with a nonsignificant reduced risk of breast cancer-specific mortality and a statistically significant reduced risk of recurrence in both U.S. and Chinese women	HR: 0.87; 95% CI: 0.70, 1.10 for low consumption of ≥10 mg isoflavones HR: 0.83; 95% CI: 0.64, 1.07 for breast cancer specific HR: 0.75; 95% CI: 0.61, 0.92 for recurrence of breast cancer

HR: hazard ratios; 95% CI: 95% confidence interval; RR: relative risk.

consumption of soy protein also decreased breast cancer risk by 54 as compared with the lowest intake. The inverse association between highest intake of soy isoflavone/soy protein with the breast cancer risk was statistically significant in postmenopausal women. In the ER/PR status stratified analysis, a significantly reduced risk was observed for ER^+/PR^+ breast cancer among highest intake of soy isoflavone and soy protein. Two other studies [74,75] also showed that the soy food intake is associated with longer survival and low recurrence among breast cancer patients. Dong et al. [76] conducted another meta-analysis of 18 prospective studies including 4 studies of breast cancer recurrence and 14 studies of breast cancer incidence through 2010. This analysis revealed that soy isoflavones consumption was inversely associated with risk of breast cancer incidence. However, the protective effect of soy was only observed among studies conducted in Asian populations but not in Western populations. Soy isoflavones intake was also inversely associated with risk of breast cancer recurrence. This study suggests that soy isoflavones intake is associated with a significant reduced risk of breast cancer incidence in Asian populations, but not in Western populations. Nechuta et al. [77] prospectively evaluated the association between post-diagnosis soy food consumption and breast cancer outcomes among U.S. and Chinese women using data from the After Breast Cancer Pooling Project. The analysis included 9514 breast cancer survivors with confirmed invasive breast cancer between 1991 and 2006 from 2 U.S. cohorts and 1 Chinese cohort. This study revealed that isoflavone consumption was inversely associated with recurrence among both U.S. and Chinese women, regardless of whether data were analyzed separately by country or combined. This large study of combined data on U.S. and Chinese women revealed that post-diagnosis soy food consumption of $\geq$10 mg isoflavones per day was associated with a non-significant reduced risk of breast cancer-specific mortality and a statistically significant reduction in risk of recurrence. Another meta-analysis of epidemiological studies of soy consumption and breast cancer risk among European women also have indicated that soy foods consumed at levels comparable to those in Asian populations have, in some cases, a significant reduction in breast cancer risk and that women who are at increased risk of breast cancer due to polymorphisms in genes associated with the disease may especially benefit from high soy isoflavone intake [78]. Altogether, the above described epidemic studies have suggested that the intake of soy products may protect against the occurrence of breast cancer because of the considerable amount of isoflavones they contain.

5. Possible molecular mechanisms of chemopreventive effects of genistein on breast cancer

Soy isoflavones are structurally similar to endogenous estrogens (Fig. 4) and may affect breast cancer through both hormonally mediated and non-hormonally related mechanisms. Among soy isoflavones, genistein (GEN) is believed to be a potent chemopreventive agent for breast cancer and thus, more studies have been focused on its chemopreventive effects on breast cancer cells. Recent studies have suggested that GEN can enhance the anticancer capacity of an estrogen antagonist, TAM, especially in ERα-positive breast cancer cells. The chemopreventive effects of GEN could be mediated via the several pathways and mechanisms.

5.1. GEN as an agonist of ERβ and its effects on endogenous estrogen metabolism

Endogenous estrogens (e.g. 17β-estradiol, E_2) synthesized in the body have significant impact on gene regulation by activating ERs in diverse cell types. While E_2 plays very important roles in the growth and development of breast, prolonged exposure to excess E_2 and/or exogenous estrogen-like chemicals has been known to be the key risk factor for the development of breast cancer. Due to its structure similarity to E_2, GEN possesses estrogenic properties and can compete with E_2 for binding to ERs. Affinity of GEN is much higher toward ERβ than toward ERα [79]. GEN can serve as either an agonist or antagonist for ERs, depending on exogenous E_2 levels. At higher exogenous E_2, GEN may act as the ER antagonist whereas at lower exogenous E_2, it may act as agonist.

Several *in vitro* and *in vivo* studies [80–82] have shown that GEN is capable of stimulating growth of some ER-containing breast cancer cells. It has been found that GEN is able to increase proliferation of estrogen-dependent breast cancer cells when it is not co-treated with an estrogen antagonist. For example, Ju et al. [80] suggested that consumption of products containing GEN might not be safe for postmenopausal women with estrogen-dependent breast cancer. These effects of GEN could be completely abolished by co-treatment of MCF-7 cells with either ICI 182780 or TAM, the estrogen antagonists. GE has been found to decrease efficiency of TAM and letrozole, drugs commonly used for breast cancer therapy [83,84] and to inhibit immune response toward cancer cells [85].

In addition to their effects on ERs, soy isoflavonoid have been shown to cause effects on endogenous estrogen metabolism. Wood et al. [86] evaluated the effects of dietary soy isoflavonoids on endogenous estrogen metabolism in a postmenopausal primate model. The results of this study suggest that long-term exposure to soy isoflavonoids, equol in particular, may facilitate clearance and catabolism of endogenous estrogen to more benign 2-hydroxylated metabolites.

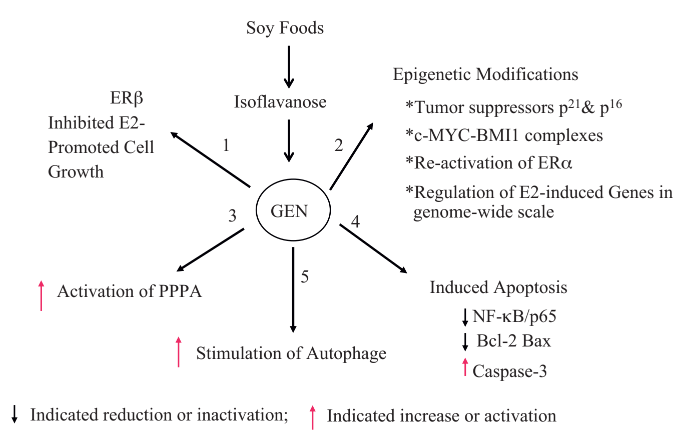

Fig. 4. Possible molecular mechanisms of chemopreventive effects of genistein on breast cancer.

As mentioned above, GEN displays weak estrogenic activity mediated by ERs with a preferential binding to ERβ. Several isoforms of ERβ, namely ERβ1, ERβ2, ERβ3, ERβ4 and ERβ5, are generated by alternative splicing [87,88]. Cappelletti et al. [89] investigated the interaction between GEN and the various isoforms of ERβ in two human breast cancer cell lines, T47D and BT20. They evaluated the effect of GEN individually and in combination with E_2 on mRNA expression of ERβ isoforms evaluated by a triple primer RT-PCR assay. In T47D cells, E_2 caused a 6-fold increase in total ERβ and modified the relative expression pattern of the various isoforms, i.e. upregulating the ERβ2 but down-regulating the ERβ5 isoform. GEN up-regulated ERβ2 and ERβ1 in T47D cells, and after GEN treatment, the ERβ2 isoform became prevalent. While in BT20 cells it almost doubled the percent contribution of ERβ1 and ERβ2 to total ERβ. GEN, through the modulation of ERβ isoform RNA expression, inhibited estrogen-promoted cell growth, without interfering with estrogen-regulated transcription. ERβ and its isoforms may be involved in a self-limiting mechanism of estrogenic stimulation promoted either by the natural hormone or by weaker estrogen agonists like GEN. Thus, by regulating the functions of ERβ, GEN confers protective effects in breast cancer cells.

5.2. *Epigenetic and genome-wide effects of GEN*

Epigenetics refers to functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence of the gene. Epigenetic modifications include DNA methylation and histone modification. Gene expression can be controlled through the action of repressor proteins that bind silencer regions of the DNA. These changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations. However, there is no change in the underlying DNA sequence of the organism, instead, non-genetic factors cause the organism's genes to express themselves differently [90,91].

An important mechanism by which GEN inhibits breast cancer is related to its potential impacts on epigenetic processes [92]. In fact, several recent studies have reported that GEN confers the epigenetic effects. For instance, Li et al. [92] investigated the impacts of GEN on epigenetic regulation during breast tumorigenesis. They examined the effects of GEN on epigenetic alterations of two key tumor suppressor genes, p21(WAF1)(p21) and p16(INK4a)(p16), and two tumor promoting genes, BMI1 and c-MYC, in an breast cancer transformation system. Their results have shown: 1) GEN significantly inhibited growth of precancerous breast cells and breast cancer cells dose-dependently but caused little effect on normal human mammary epithelial cells; 2) GEN up-regulated expression of p21 and p16 whereas down-regulated expression of BMI1 and c-MYC; 3) GEN treatment altered histone modifications in the promoters of p21 and p16 and the binding ability of the c-MYC-BMI1 complexes to p16 promoter, contributing to GEN-induced epigenetic activation of these genes; and 4) an orally-fed GEN diet prevented breast tumorigenesis and inhibited breast cancer development in breast cancer mice xenografts. These results suggest that GEN may repress early breast tumorigenesis by epigenetic regulation of p21 and p16 through impacting histone modifications and the BMI1-c-MYC complex recruitment to the regulatory region in their promoter regions.

Another study revealed that the epigenetic reactivation of ERα by GEN enhanced hormonal therapy sensitivity in ERα-negative breast cancer. It has been known that DNA methylation of the promoter region of ERα is an epigenetic mechanism by which the abundance of ERα mRNA is regulated and a differentially methylated single CpG-site is involved in ERα transcription [93]. Inactivation of ERα due to the methylation of the promoter region B of ERα occurs in part of breast cancer cells, i.e. ERα-negative breast cancer cells [94]. Another study [95] revealed that the degree of ERα gene promoter methylation correlated with ERα expression and epigenetic alteration of ERα gene may play an important role in the pathogenesis of familial breast cancer patients among Chinese women. Li et al. [96] investigated the *in vitro* and *in vivo* epigenetic effects of GEN on ERα reactivation and found that GEN reactivated ERα expression in ERα-negative MDA-MB-231 breast cancer cells and that this effect was synergistically enhanced when GEN was combined with, a histone deacetylase (HDAC) inhibitor and trichostatin A. GEN treatment also re-sensitized ERα-dependent cellular responses to E_2 and TAM. Further studies revealed that GEN can lead to remodeling of the chromatin structure in ERα promoter, contributing to ERα reactivation. Consistently, GE significantly prevented cancer development and reduced the growth of ERα-negative mouse breast tumors. Dietary GEN further enhanced TAM-induced anti-cancer efficacy due, at least in part, to epigenetic reactivation of ERα. The results reveal a novel therapeutic combination approach using bioactive soybean products, e.g. GEN and anti-hormone therapy in refractory ERα-negative breast cancer, which will provide more effective options in breast cancer therapy.

GEN also has an impact on gene expression at a genome-wide scale. Gertz et al. [97] performed chromatin immunoprecipitation experiments followed by sequencing (ChIP-seq) to identify ERα binding sites, and RNA-sequence in endometrial cancer cells treated with GEN or E_2. GEN induced thousands of ERα binding sites and expression changes of more than 50 genes, representing a subset of E_2-induced genes. Genes affected by E_2 were highly enriched for ribosome-associated proteins whereas GEN failed to regulate most ribosome-associated proteins and instead enriched for transporters of carboxylic acids. Treatment-dependent changes in gene expression were associated with treatment-dependent ERα binding sites. GEN exhibited a similar relationship to E_2 in the breast cancer line T-47D, where cell type specificity played a much larger role than treatment specificity. Overall, GEN clearly regulates gene expression through ERα on a genome-wide scale, although with lower potency resulting in less ERα binding sites and less gene expression changes compared to those induced by E_2. Zhang et al. [98] treated five-week-old mouse mammary tumor virus-erbB2 female transgenic mice with low-, normal- and high-levels of estrogen and then gave each group of animals with soybean feed or with control feed. Their results suggest that dietary soy isoflavones promote breast cancer at low estrogen levels but inhibit breast cancer at high estrogen levels. This effect may only occur during the initiation stage of breast cancer.

5.3. Effects of GEN on activation of peroxisome proliferator-activated receptors (PPARs)

Peroxisome proliferator-activated receptors (PPARs), a group of transcription factors, are involved in regulation of a large number of genes [99] and play essential roles in the regulation of cellular differentiation, development, and carbohydrate, lipid, and protein metabolisms, and tumorigenesis of higher organisms [100–102]. Three forms of PPARs, namely PPARα, PPARβ/δ and PPARγ, which are transcribed from different genes, are known. GEN is capable of binding and activating all three PPAR isoforms, α, δ, and γ. GEN can directly interact with the ligand binding domain of the PPARγ with a Ki being 5.7 mmol/L [103]. At concentrations ranging from 1 to 100 mol/L, GEN activated PPARs in breast cancer MCF-7 cells, T47D cells and MDA-MD-231 cells in a dose-dependent manner. Several studies have shown that both ERs and PPARs influenced each other and therefore induce differential effects in a dose-dependent manner and the final biological effects of GEN are determined by the balance among these pleiotrophic actions [104,105].

5.4. Effects of GEN in induction of apoptosis

Apoptosis is the process of programmed cell death (PCD) characterized by blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation [106]. It has been known that GEN can stimulate cell growth activity at nanomolar concentrations but inhibits cell growth at micromolar concentrations. GEN inhibits cell proliferation and triggers apoptosis in human breast cancer cells and triple negative breast cancer cells (TNBC), which lack ERα, PR and overexpression of human epidermal growth factor receptor 2 (HER2). Shim et al. [107] observed that treatment of MCF-7 cells with GEN at 100 μm/L effectively induced apoptosis via activation of calpain, caspase 7 and poly (ADP ribose) polymerase and increased phosphorylation of p38 mitogen-activated protein kinase, and increased phosphorylation of apoptosis signaling kinase 1. It has been known that GEN in combination with photoactivated hypericin suppressed the expression of Bcl-2 and Akt in human breast cancer cells [108].

It is worth noting that GEN causes inhibitory effects on MDA-MB-231 cells, a typical TNBC [109,110]. NF-κB signaling pathway is involved in regulation of a number of important biological processes including proliferation, differentiation, and apoptosis by regulating the transcription of target genes and is a commonly active pathway in TNBC [111]. Treatment of these cells with GEN caused a dose-dependent decrease in NF-κB/p65 protein levels and DNA-binding activity of NF-κB, down-regulation of Bcl-2, a pro-apoptotic protein and up-regulation of Bax, an apoptotic protein. Because the promoter of Bcl-2 contains NF-κB binding sites, GEN might inhibit the expression of Bcl-2 via down-regulation of NF-κB. Treatment of MDA-MB-231 cells with GEN resulted in cleavage of caspase-3 and induction of caspase-3 activity in a dose-dependent manner. GEN inhibited NF-κB activity via the MEK5/ERK5 pathway and cell growth, and induced apoptosis, indicating that inhibition of the MEK5/ERK5/NF-κB pathway may be an important mechanism by which GEN suppresses cell growth and induces apoptosis in TNBC. Pan et al. [110] observed that treatment of MDA-MB-231 cells with GEN resulted in G2/M phase accumulation of cells. These results suggest that inhibition of NF-κB activity via the Notch-1 pathway may be a mechanism by which GEN suppresses the growth of TNBC.

5.5. Role of GEN in stimulation of autophagy

Autophagy is the basic catabolic mechanism involved in cell degradation of unnecessary or dysfunctional cellular components through the lysosomal machinery. The breakdown of cellular components can ensure cellular survival during unfavorable conditions such as starvation by maintaining cellular nutritional and energy levels [112]. Proper regulation of autophagy ensures the synthesis, degradation and recycling of cellular components [113]. Autophagy is another form of PCD, which suppresses tumorigenesis [114]. Thus, dysregulation of autophagic pathways has recently been implicated in the pathogenesis of a number of diseases including cancers [112,115]. It has been reported that GEN induced both apoptosis and autophagy in several types of cancer cells including ovarian cancer cells, human colon cancer HT-29 cells and lung cancer cells [116–119]. It has been known that autophagy also plays an important role in breast cancer [110–121]. It is likely that GEN is able to stimulate autophagy in breast cancer cells and the precise roles of GEN in induction of autophagy in breast cancer cells merit further investigations.

6. Summary and prospective

In this work, we reviewed the available information about the consumption of soy foods, soy isoflavones and breast cancer incidence. First, we described briefly the history of soybean and current status of soybean production as well as several types of soy products and their nutritional functions and consumption. There is an increasing trend in production and consumption of soy foods in USA and around the world during the last decades. Several lines of epidemiological evidence indicate a linear relationship between increasing soy consumption and a decreased risk of recurrence and/or mortality of breast cancer, particularly among Chinese women. The possible molecular mechanisms involved in the chemo-protective effects of GEN on breast cancer include the effects of GEN as an agonist of ERβ, epigenetic and genome-wide effects, activation of PPARs, induction of apoptosis and stimulation of autophagy. However, the precise molecular mechanisms are still far from being clearly understood. Given that Chinese women have the tradition of consumption of more soybean foods, which is related to lower breast cancer incidence and that there is an increasing trend of breast cancer incidence in China, partially due to the recent switch of traditional dietary styles to the “Westernized” styles, it is important to conduct further in depth and more comprehensive studies on the molecular mechanisms underlying the protective effects of soybean isoflavones on breast cancer particularly in Chinese women. These studies would be valuable for paving a scientific basis for future prevention and/or slowing down the increasing trend of breast cancer in China by switching current “Westernized” dietary styles back to the traditional dietary styles of consumption of more soybean-derived foods.

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