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Legume-Derived Bioactive Compounds for the Prevention and Treatment of Breast Cancer

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1. Introduction

Breast cancer is one of the most prevalent cancer types among women worldwide (Jemal et al., 2011); however, its incidence rates among populations are heterogeneous. Epidemiologic studies have shown that breast cancer incidence in Asian women is 40% lower than in Caucasian women (Goldin et al., 1986). A reasonable explanation for the difference in the cancer incidence rates could be related to intrinsic biological characteristics present in each population. For example, in general, breast cancer growth requires the presence of estrogen and it is known that Asian women have lower estrogen serum levels than Caucasian women (Shimizu et al., 1990). Nevertheless, epidemiologic studies have reported that when Asian women moved to western countries the breast cancer incidence of their subsequent generations were similar to the Caucasian women (Wu et al., 1996). Therefore, it seems that other factors have been influencing breast cancer incidence rates in each population.

In fact, it is known that only 10-15% of all breast cancers cases are caused by genetic predisposition such as BRCA and Li-Fraumeni mutations; whereas the remaining 85-90% of cases are attributed to environmental, reproductive, lifestyle factors including radiation, chemicals, late pregnancy, early menarche, nulliparity, diet and reduced physical activity (Colditz et al., 1995). The diet is an important factor affecting breast cancer incidence rates and is estimated to be correlated with 50% of new diagnosed cases (Willett, 1995). It has been described that diets based on the consumption of garlic, onion, tomato, vegetables, fruits and legumes are associated with reduced breast cancer risk. One of the major differences between western and Asian populations is their diet. The consumption of legumes (soy, beans, peas) in Asian populations is expressively higher than in western populations. These disparities on breast cancer risk and on legume intake have attracted the attention of scientists and since then this topic has been the goal of innumerable researches (Messina et al., 2006).

In addition to their importance as a nutritive food source, legumes and their bioactive compounds have also been described to show protective and therapeutic effects not only in breast cancer, but also in symptoms of menopause, heart disease and osteoporosis. On the other hand, findings suggesting no effects or possible risks in legume intake and breast cancer have also been published (Messina et al., 2008). Therefore, the evaluation of the effects of legume consumption on women at high risk for breast cancer and breast cancer patients is an important public health goal (Messina et al., 2006). In this chapter, we provide a comprehensive review of the biological, nutritional and economic background on legumes

and gather the current knowledge regarding the benefits and risks of their bioactive molecules in breast cancer prevention and treatment.

2. Legumes - biological, nutritional and economic aspects

The legumes are classified in the family Fabacea (or Leguminosae) – including around 700 legume genera and 20,000 species – and are the third largest flowering plant family (Doyle et al., 2003; Gepts et al., 2005). They present a large range of variation and are also well adapted to several temperatures and climates (Doyle et al., 2003). Despite the large number of species, only a few legumes are generally known due to their use as feeds and foods. Clovers (*Trifolium* sp.), vetches (*Vicia faba*) and alfafa (*Medicago sativa*) are mainly grown for animal feeding; while beans (*Phaseolus vulgaris*), soybeans (*Glycine max*), lentils (*Lens esculenta*), peas (*Pisum sativum*), and peanuts (*Arachis hypogaea*) are the main species grown for food (Doyle et al., 2003; Gepts et al., 2005).

Legumes nutritional profile includes dietary fibers, low glycemic indexes, no cholesterol, low levels of fat (2- 5%), and high amounts of carbohydrates (55- 60%). In addition, essential minerals and vitamins for human health are also present (Rochfort et al., 2007). High protein content (20-40%) is another notable feature of legumes which is known to be 2-3 times higher than cereals. Along with their high protein content, they also produce a good balance of all essential aminoacids, with the exception of methionine (Rochfort et al., 2007).

Among legumes, soybeans have not only the highest protein content but also the highest protein digestibility, which is typically 90%. Soybeans and peanuts are considered an exception among legumes in terms of nutritional profile. Despite producing high protein contents, they are low in carbohydrates but high in fat (40% total energy) (Messina et al., 2010). In addition, soy is a component widely used to fortify school breakfast and lunch programs and is also present in upwards 60% of processed foods (Patisaul et al., 2010).

Some legumes produce antinutritional factors which have shown to induce allergy and intestinal disturbance when eaten raw. However, the majority of these toxins can be eliminated through heating or other industrially processing (Gepts et al., 2005).

The intake rates of legumes vary dramatically among populations worldwide. In Asian countries, legumes have been consumed for centuries representing ~50% of their diet which achieve and even surpass the minimum intake recommended. On the other hand, the rates of legume consumption by North American and European countries are low (Messina, 2010).

A few decades ago researchers have reported that some compounds produced by legumes could promote protective and therapeutic effects on human health. This new vision of legumes as functional foods has induced a profound impact in sales and consumption of legumes in countries which had low consumption rates as the EUA and European countries (Messina et al., 2001; Messina, 2010; Patisaul et al., 2010).

3. Legume bioactive compounds and breast cancer

Hypothesis regarding protective and therapeutic effects of legumes in breast cancer have been formulated mainly based on epidemiological studies suggesting a negative correlation between legume intake and breast cancer incidence among populations worldwide. Asian women, who traditionally consume high amounts of legumes daily, are 4 to 10 times less likely to be diagnosed with and die from breast cancer than are people in the United States

(Fournier et al., 1998). Interestingly, genetic variations do not seem to be the main factors involved in this disparity since Asian women who immigrate to the United States and adopted a "Western" lifestyle, particularly a diet poor in legumes, develop breast cancer risk comparable to Caucasian women within two generations (Wu et al., 1996).
These evidences have triggered several researches seeking for the identification of potential legume molecules involved in breast cancer prevention and treatment. Isoflavones, protease inhibitors and peptides are the main legume bioactive compounds evaluated in this field. In the next sections, their anticancer properties as well as synergistic effects are discussed.

4. Isoflavones

Legumes produce large amounts and several isoflavones isoforms which are assumed to have antimicrobial activity and to play an important role in plant protection (Rochfort et al., 2007). In particular, soybeans produce 12 isoforms of isoflavones and contain the highest dietary-relevant amounts of these compounds among legumes (Franke et al., 1998). For example, each gram of soy protein in soybeans contains approximately 3.5 mg of isoflavones; while no significant amounts are present in lentils (Murphy et al., 1999). For this reason, the majority of published data regarding their activities in breast cancer involve isoflavones found in soy.

4.1 Structural and bioavailability

The isoflavones are a subclass of flavonoids and belong to the group of naturally occurring heterocyclic phenols. Their basic structure is composed of 2 benzene rings linked through a heterocyclic pyrane ring. Isoflavones are named glycoside (inactive form) when conjugated to glucose or carbohydrate moieties and glycone (active form) when unconjugated (Franke et al., 1998) (Fig.1).

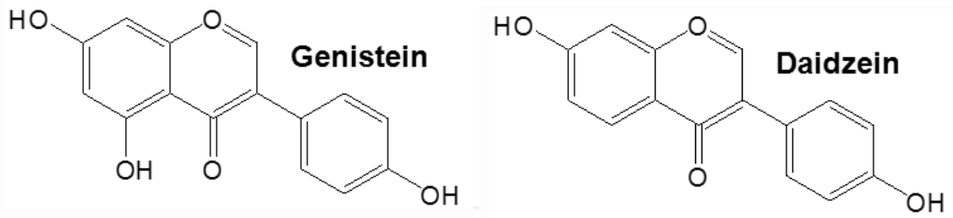

Fig. 1. Chemical structures of the genistein (4', 5, 7-trihydroxyisoflavone) and daidzein (4', 7-dihydroxyisoflavone), the most abundant isoflavones found in soy. The figure was drawn by ChemDraw (Cambridge Soft, version 9.0).

The primary isoflavones found in soybeans are the glycones genistein (4',5,7-trihydroxyisoflavone), daidzein (4',7-dihydroxyisoflavone) and glycitein (7,49- dihydroxy-6-methoxyisoflavone), and their respective glycosides genistin, daidzin and glycitin (Messina et al., 2001). Genistein and daidzein are the most abundant isoflavones in soybeans representing 50% and 40% of the total isoflavone content respectively (Rochfort et al., 2007).
The majority of isoflavones found in raw soybeans are almost entirely as glycosides (genistin, daidzin, and glycitin) while only 1 – 3% account for their active form glycone (Murphy et al., 1999). After ingestion, they are rapidly absorbed entering systemic circulation predominantly as conjugated forms (95%) with limited bioavailability (Messina

et al., 2006). Isoflavones then are further deconjugated by the action of glucosidases produced in intestinal bacteria. Interestingly, there is considerable inter-individual variation in intestinal bacteria metabolism of genistein and daidzein. The bioconvertion of daidzein to one of its metabolites (equol) is performed by a very specific type of intestinal bacteria which have been found only in 30–50% of individuals (Patisaul et al., 2010).
The resulting isoflavone metabolites are widely biodistributed and their serum levels can reach the low micromolar range according to the amount ingested (Messina et al., 2001). Soy isoflavones have half-lives of approximately 8 hours and are nearly all excreted within 24 hours after consumption (Messina et al., 2006).

4.2 Exploring isoflavones biological effects on breast cancer

4.2.1 Estrogen-receptor dependent properties

Breast cancer development is significantly influenced by the exposure to estrogens. These hormones have been described to induce proliferation of malignant breast cells contributing to breast cancer promotion and progression. Therefore, the control of estrogen exposure is a key factor in breast cancer chemoprevention (Bouker et al., 2000).
The structures of soy isoflavones are similar to mammalian estrogens (Messina et al., 2001). By the early 1960s, isoflavones were characterized as ligands of estrogen receptors and thus labelled as phytoestrogens (Cheng et al., 1953). These findings led to an initial enthusiasm mainly based on the possibility that isoflavones might exert antiestrogenic effects on breast tissue as other known estrogen antagonists such as tamoxifen (Messina et al., 2001).
Folman & Pope were the first to conduct assays establishing the relative binding affinity isoflavones for the estrogen receptor (ER) (Folman et al., 1966). There are two major ER subtypes in mammals, ER-α and ER-β presenting different tissue distributions. Soy isoflavones have singular affinities for each ER and for this reason are classified as selective estrogen receptor modulators (SERMs) (Messina et al., 2006). Genistein is 7- to 48-fold more selective for ER-β than Er-α and is 1,000-fold more potent at triggering transcriptional activity with ER-β than Er-α (Kuiper et al., 1998). However, isoflavones are considered to be weak estrogens, showing binding affinity to ER-α and ER-β of nearly 20- and 5-fold less than estradiol, respectively (Kuiper et al., 1998).
There are some evidences suggesting that activation of ER-β inhibits proliferation in breast cells (Patisaul et al., 2010). Because genistein preferentially binds to ER-β, it may induce antiestrogenic effects through this receptor (Bouker et al., 2000). Moreover, soy isoflavones can act as an antiestrogen through other mechanisms. Genistein has the ability to inhibit the enzyme 17β-hydroxysteroid oxidoreductase type 1 (HSOR-1), which is necessary for estradiol secretion from the ovaries in premenopausal women and is essential for the reduction of estrone to estradiol in the adipose tissues. Isoflavones are also involved in the inhibition of the aromatase enzyme, which is responsible for the conversion of androgens to estrone in peripheral (adipose) tissues (Bouker et al., 2000). Thus, the inhibition of estrogen–metabolizing enzymes can lead to a decreased total estradiol level and intensify isoflavones antiestrogen effects. It is important to highlight that complex feedback mechanisms associated with the hypothalamic/ pituitary/gonadal axis are involved in controlling the levels of estrogen and that the effects of isoflavones in this network are unclear and demand further studies (Bouker et al., 2000).
The main biological effects of soy isoflavones in breast cancer cells involve cell growth arrest and induction of apoptosis (Lamartiniere et al., 1998). Genistein have been shown to inhibit

growth factor- and cytokine -stimulated growth of breast cancer cells (Peterson et al., 1996). Indeed, at the molecular level, this isoflavone can influence the regulation of cell cycle molecules by inducing significant up-regulation of p21/WAF1 expression (cell cycle inhibitor) in the treatment of breast cancer cells (Banerjee et al., 2008). In addition, the treatment of breast cancer cells with genistein influences the regulation of apoptotic molecules such as down-regulating anti-apoptotic molecules (Bcl-2, Bcl-xL, and HER-2/neu) and up-regulating pro-apoptotic ones (Bax and caspases). It has been suggested that genistein could also induce the regulation of those genes through the inhibition of proteasome. Overall, these findings suggest that ER stress, cell cycle arrest and apoptosis induction may represent part of the molecular mechanism by which isoflavones exert their anticarcinogenic effects (Banerjee et al., 2008).

4.2.2 Estrogen-receptor independent properties

Interestingly, the effects of soy isoflavones on cell cycle arrest and apoptosis has been detected not only in ER-positive but also in ER-negative breast cancer cells (Theil et al., 2010) suggesting that isoflavones anticancer activity might also occur independently of ER modulation. Indeed, several non-estrogenic targets for isoflavones have been described.

Isoflavones have been described as specific inhibitors of protein-tyrosine kinase (PTK), which is an enzyme frequently overexpressed in cancer cells. PTKs are crucial molecules for tumor development and thus soy isoflavones can potentially slow tumorigenesis by inhibiting their mediated signalling mechanisms (Banerjee et al., 2008; Patisaul et al., 2010).

Isoflavones can modulate and block the activity of several molecules involved in breast cancer cell growth and survival pathways such as topoisomerase I and II, mitogen activated protein kinases (MAPK), urokinase- type plasminogen activator (uPA), and nuclear factor-κB (NF-κB). Isoflavones are also implicated in the growth inhibition of various cancer cells through the regulation of gene activity by modulating epigenetic events that are intimately related to the regulation of cell cycle and apoptosis such as DNA methylation and/or histone acetylation (Messina et al., 2001; Banerjee et al., 2008).

Furthermore, soy isoflavones are able to inhibit invasion, metastasis, and angiogenesis *in vitro* and *in vivo* in a number of cancers including breast cancer. Genistein was described to inhibit the secretion of matrix metalloproteinases (crucial enzymes for invasion and metastasis) in MDA-MB-435 breast cancer cells and blocked invasion of a highly meta-static subline of BALB/c mammary carcinoma cells (Bouker et al., 2000; Messina et al., 2001).

Antioxidant activity is also included among the described effects of soy isoflavones. They are able to protect cells against reactive oxygen species by scavenging free radicals and inhibiting the expression of stress–response related genes which is an interesting approach for cancer prevention (Ruiz-Larrea et al., 1997).

Overall, it is clear that when evaluating biological effects of isoflavones it is necessary to look beyond the estrogen receptor and consider their non-hormone-related activities (Messina et al., 2001).

4.2.3 Dose-dependent effects

Soy isoflavones can induce different effects on breast cancer cells according to the dose used. For example, the effects of soy isoflavones in MCF-7 and BT20 breast cancer cells were only observed in the highest dose tested (50 μg/mL) (Theil et al., 2010). Similar effects were reported showing that isoflavone doses higher than 10 μM could inhibit the growth of breast

cancer cells (Wang et al., 1996). In particular, the IC50 values of genistein able to induce growth arrest in both hormone-dependent and hormone-independent breast cancer cells have been described to range from 10 to 50 μM (Messina et al., 2001).

Some researchers question the relevance of these results by claiming that the high isoflavone concentrations used *in vitro* would not be achieved after ingestion in an *in vivo* system. Most Asians or Caucasians that consume a diet rich in soy have serum genistein levels smaller than 1 μM (Bouker et al., 2000). In order to address these claims, several assays evaluated the effects of isoflavones in low concentrations and, surprisingly, showed a different outcome. Low doses (0.01–1 μM) of genistein were shown to stimulate proliferation in human breast cancer cell lines (Bouker et al., 2000; Messina et al., 2001).

In animal studies, soy isoflavones also induce different activities according not only to the dose but to the animal model and the route of administration used (Barnes et al., 1997). Injections of 0.8 mg genistein in rats significantly reduced MNU-induced tumor multiplicity and marginally reduced tumor incidence. Similarly, a high dose of daidzein (0.8 mg) decreased tumor multiplicity without affecting incidence, whereas a low dose (0.4 mg) was ineffective. Other study showed that rats fed with a low dose of biochanin A (10 mg/kg), which is an isoflavone that is converted to genistein *in vivo*, significantly reduced tumor multiplicity and that a higher dose (50 mg/kg) also reduced tumor incidence (Barnes et al., 1997).

Conversely, instead of decreasing tumor growth as previously commented, soy isoflavones have also been described to stimulate breast tumor growth *in vivo* (Helferich et al., 2008; Bouker et al., 2000; Allred et al., 2004). The effects of dietary level of genistein were studied in an athymic BALB/c ovariectomized mice model subcutaneously injected with human estrogen-dependent cells (MCF-7). They found that in mice fed with a standard (control) AIN-93G diet, tumors reduced completely; however, mice fed with diets containing either isoflavone-rich isolated soy protein or isoflavone extracts had tumor growth stimulated (Hsieh et al., 1998). The use of a non-ovariectomized athymic mice model injected with ER-negative breast cancer cells to evaluate the effects of high dietary levels of soy isoflavones intake showed that daidzein increased while genistein decreased mammary tumor growth by 38 and 33% respectively. In addition, daidzein increased lung and heart metastases while genistein decreased bone and liver metastases. Combined soy isoflavones did not affect primary tumor growth but increased metastasis to all organs tested, which include lung, liver, heart, kidney, and bones (Martinez-Montemayor et al., 2010).

In general, the evidences indicate that at lower concentrations isoflavones exert estrogen-like effects while at higher concentrations other non-estrogen receptor–mediated effects are induced (Messina et al., 2001). Data from *in vivo* studies suggest that in a low-estrogen environment (as exists in postmenopausal women), genistein is estrogenic and has a proliferative effect on breast tissue. However, in a high estrogen environment (similar to that in premenopausal women), it has an antiproliferative and possibly antiestrogenic effect (Hsieh et al., 1998; Allred et al., 2004). Therefore, isoflavones can both inhibit or stimulate proliferation of breast cancer cells showing a biphasic effect according to the dose (Bouker et al., 2000).

Several hypothesis and discussion about experimental models limitations/weaknesses have been elaborated to address these conflicting results (Messina et al., 2008). First, considering that both estrogen and soy isoflavones bind to ER, differences in endogenous estrogen levels may interfere in the results. Premenopausal women have high levels of estrogen while basal levels of estrogen are found in postmenopausal women. Thus, *in vitro* estrogen-depleted

conditions and ovariectomized animals (with no basal estrogen levels) would not be suitable models because there are no sufficient estrogen levels to promote or even to maintain estrogen-dependent tumors. Even weak estrogenic compounds, such as isoflavones, could stimulate the growth of estrogen-sensitive mammary tumors in such environment (Messina et al., 2006). Thus, these models would not accurately reflect conditions in either premenopausal or postmenopausal women (Messina et al., 2008). Researchers have supported this hypothesis showing that although genistein stimulated proliferation of MCF-7 cells and enhanced expression of the estrogen-responsive pS2 gene in an estrogen-depleted *in vitro* environment, it inhibited estrogen-induced proliferation and reduced pS2 expression of MCF-7 breast cancer cells when in the presence of a maintained level of estrogen (Wang et al., 1996; So et al., 1997). Nevertheless, breast tumor growth stimulation by both dietary and subcutaneously injected genistein has still been noted in animal models in which estrogen levels were more similar to the amounts of pre and postmenopausal women (Messina et al., 2006).

The second critique addresses the use of mice lacking the immune properties (athymic or nude). This animal feature is a necessary element of these models in order to allow the growth of human tumor cells in a murine environment. However, the lack of immune function may eliminate a potential mechanism by which soy isoflavones reduce tumor development (Messina et al., 2008). A recent research in B6C3F1 mice showed that enhanced immune function resulting from pre-treatment with genistein (20 ppm) is correlated with protection against chemically-induced mammary tumors (Guo et al., 2007).

The third critique relates to isoflavone dose. In many studies, breast cancer cells and animals are exposed to high amounts of genistein (750 ppm) which exceeds typical dietary intake. In Japan, adults consume about 15–20 mg genistein daily (total average isoflavone intake is approximately 40 mg), which equates to a dietary concentration of about 30–40 ppm (Messina et al., 2008). However, it is important to highlight that isoflavone biodistribution is not homogeneous. Isoflavone concentrations in breast tissue are two- to threefold higher than paired serum concentrations. It suggests that breast tissue may be exposed to higher levels of biologically active isoflavones than was previously thought (Pasqualini et al., 2005) and supports investigations of high concentrations of isoflavones.

Another aspect of oral doses of isoflavones relies in the amount of free (unconjugated) isoflavones processed by intestinal bacteria. The rodent gut bacteria are able to convert daidzein to the metabolite equol more effectively than humans. Furthermore, even in humans who are classified as equol producers, genistein is the predominant serum isoflavone in response to the ingestion of soy or mixed isoflavones, whereas equol predominates in most other species, including both rodents and monkeys (Gu et al., 2006).

The fourth consideration is based on the fact that it is not clear to what extent the existing MCF-7 xenoplants in nude mice resemble tumors in human breast cancer. These tumors are fully transformed and composed of cells that are extremely sensitive to the growth-stimulating effects of estrogen (Messina et al., 2008). Thus, a better comprehension of the current existing animal models and the development of new ones would contribute to the interpretation and translation of isoflavone effects in humans.

Given the conflicting data and limited *in vitro* and *in vivo* models, the controversy about the effects of isoflavones either from soy foods or supplements would be unlikely solved by additional animal research (Messina et al., 2009). Then, epidemiologic data should be another alternative to study and conclude about isoflavone intake and breast cancer. Current data discussing this topic is provided in the next section.

4.2.4 Aspects of isoflavone intake in humans

Women with high risk of breast cancer, breast cancer patients and survivors are among the group of consumers who have embraced soy products, isoflavone supplements and isoflavone-enriched foods, seeking for their health-promoting properties. Nevertheless, the estrogenic/antiestrogenic effects of these molecules and the disparities of *in vitro* and *in vivo* data have led to considerable controversy and misinterpretation among health professionals and consumers over the use of soy by this group of women. Due to the phytoestrogenic nature of isoflavone, several oncologists often discourage and even prohibit its intake by breast cancer patients, particularly those with ER-positive tumors (Messina et al., 2001).

As previously discussed, early epidemiologic studies have reported that high isoflavone intake was related to low cancer rates regardless of intrinsic genetic and biological differences among populations worldwide (Wu et al., 1996). Since then, more researchers have attempted to refine the knowledge of this matter and further investigate the correlations of isoflavone intake among breast cancer biomarkers, time of exposition, and age.

Soy intake was found to be significantly associated with a decreased risk of death from breast cancer and/or recurrence when evaluated in 5,042 Chinese women aged from 25 to 75 followed for 5 years (Shu et al., 2009). These benefits remained significant even after adjusting the results for 17 factors including tumor, node, metastasis stage, ER, progesterone receptor status and the type of treatment received. It was also observed that women who had the highest level of soyfood intake and did not take tamoxifen had a lower risk of mortality and a lower recurrence rate than women who had the lowest level of soyfood intake and used tamoxifen (Shu et al., 2009; Messina et al., 2010). The association of decreased breast cancer risk and soy isoflavone intake has been observed even in Asian-American women, both pre- and postmenopausal, living in the West (Wu et al., 1996). Overall, evidences show that for Asian women the risk of developing breast cancer reduces as soy intake rises. Even a soy intake of as little as 10 mg per day was sufficient to decrease breast cancer risk by 12% (Patisaul et al., 2010).

Effects of isoflavone intake have also been investigated in non-Asian populations. In a US study, it was observed that breast cancer survivors (n= 1,954; followed for 6.3 years) had reduced risk of cancer recurrence with increasing amounts of isoflavone among postmenopausal women and tamoxifen users. Interestingly, more pronounced effects were observed in women with ER-positive breast cancer (Guha et al., 2009). Beneficial effects of isoflavone intake were also observed in a Dutch study, which compared serum isoflavone concentrations in women with and without breast cancer. It was observed that high plasma concentrations of genistein were associated with a 32% reduction in breast cancer risk (Verheus et al., 2007). Furthermore, it was reported that isoflavone intake was associated with a reduced risk of all-cause mortality during the 5-y follow-up period among postmenopausal U.S. breast cancer patients (Fink et al., 2007).

Conversely, other investigations have failed in detecting benefits in soy isoflavone intake. One of them did not find significant differences in soy daidzein or genistein intake between breast cancer cases and their controls in Shanghai (Zheng et al., 1999). Other investigation showed that soy food intake was unrelated to survival of Chinese breast cancer patients during the 5.2-y follow-up period (Boyapati et al., 2005). Several other researchers have also suggested that soy consumption is not associated with a reduced risk of breast cancer; however, no harmful effects were found in these studies (Bouker et al., 2000).

Breast tissue density has been used as a non-invasive breast cancer biomarker to evaluate isoflavone intake. It was observed that both intervention and epidemiologic studies have not

shown evidence of neither harm or benefit of isoflavone on breast cancer density (Messina et al., 2006). Analysis of breast cell proliferation has also been used as a biomarker of potential tumor promotion. Comparison of biopsies taken before and after exposure to soy products did not show increased cell proliferation in any of the four different trials involving breast cancer patients, healthy subjects, and women undergoing breast biopsy or definitive surgery for breast cancer. Daily isoflavone intake in these trials ranged from 36 to more than 100 mg, with study periods ranging from 2 weeks to one year (Messina et al., 2009). Another study examining more than one breast cancer biomarker found no statistically significant differences in cell proliferation (Ki67 index), histology (hyperplasia with or without atypia), or ER expression in 6 and 12 months of soy intake (Messina et al., 2006). Conversely, a study evaluating the effects of soy consumption (38 g soy protein isolate, 80 mg isoflavones) over 5 months showed an association of isoflavone intake and a two- to sixfold increase in breast nipple aspirate fluid (NAF) secretion of premenopausal but not postmenopausal women (Petrakis et al., 1996).

The effects of isoflavone intake in serum estrogen and androgen levels have been widely investigated. The consumption of textured vegetable (i.e., soy) protein for 2 weeks elicited an ER-mediated response detected by increased pS2 levels (protein expressed in response to estrogen) in breast biopsies taken from premenopausal women (Hargreaves et al., 1999). However, high levels of soy products have been described to induce no changes or even decrease plasma estradiol concentrations in premenopausal women. None of these effects were observed in postmenopausal women (Bouker et al., 2000). These contrasting findings could be partially explained by the observation that, despite binding to ERs, isoflavones are also able to inhibit enzymes related to estrogen synthesis and metabolism. Therefore, it has been hypothesized that the presence of other additional simultaneous stimuli may result in either reduced or increased circulating estradiol concentrations (Bouker et al., 2000).

Isoflavone intake has been implicated to interfere in serum levels of other menstrual cycle hormones, such as progesterone. Studies have reported that isoflavone intake was associated with a significant reduction in serum progesterone levels and in luteal phase lengths (Lu et al., 2000). These findings suggest that isoflavones may reduce the probability of neoplastic transformation and breast cancer development since breast cells are more proliferative during the luteal phase of the menstrual cycle, when progesterone concentrations are the highest (Lu et al., 2000).

Overall, inconsistent results about isoflavone intake and breast cancer are also present in epidemiological studies. Indeed, researchers have highlighted the difficulties of comparing clinical studies since several variables are not properly considered. In a meta-analysis by Trock et al., 18 studies (12 case-control and 6 cohort) published between 1978 and 2004 were evaluated. After making several assumptions, the authors showed that there is a small inverse correlation between soy intake and breast cancer for both pre- and postmenopausal women; however data limitations cannot exclude the possibility that this result could be an artifact of the analysis (Trock et al., 2006).

An important variable to be considered when evaluating risks and benefits of soy isoflavone intake comprises the extent and the period of life in which women are exposed to soy food. Studies attempting to address this topic are discussed in the next section.

4.2.5 Lifetime exposition of isoflavones

Throughout the life span, estrogens can induce mammary cell proliferation or cell differentiation depending on the overall hormonal environment. Since isoflavones present

estrogenic or antiestrogenic activities, they may have a different impact on the breast if the exposure occurs in utero; during childhood, puberty, or pregnancy; premenopausally; or during postmenopause (Bouker et al., 2000). Studies have been carried out to determine if the putative preventive effects of soy isoflavones are related to the lifetime period of exposition.

Different outcomes have been shown from perinatal/neonatal exposure of isoflavones in animals. The offspring of pregnant rats receiving subcutaneously administration of high doses of genistein exhibited abnormal mammary gland development and higher susceptibility to develop 7,12-dimethylbenz[a]anthracene (DMBA)- induced mammary tumorigenesis (Padilla-Banks et al., 2006; Patisaul et al., 2010). Conversely, it was reported that rat pups born to mothers consuming high levels of genistein during gestation and lactation developed fewer breast tumors (Fritz et al., 1998). Protective effects of isoflavones were also reported when soy exposure occurred perinatally. Rats receiving genistein through diet or subcutaneously during the first days postpartum showed lower tumor incidence after DMBA mammary tumor induction (Lamartiniere, 2000).

The period between puberty and a first full-term pregnancy is when the breast is particularly vulnerable to the effects of carcinogens. During this time, there are a high percentage of indiferentiated breast cells, named terminal end buds (TEBs), actively proliferating. Several investigations in animal models have shown protective effects of prepubertal isoflavone exposure on mammary tumorigenesis induced by DMBA (Murrill et al., 1996; Lamartiniere et al., 1998).

Epidemiological studies support *in vivo* findings showing that isoflavone intake during adolescence and adulthood is correlated with low risk of breast cancer. Shu et al. reported that women consuming tofu (11 g soy protein/day) during their teenage years (13–15 years) were less likely to develop premenopausal and postmenopausal breast cancer as adults (Shu et al., 2001). Other epidemiologic studies have supported these results when protective effects with reductions in risk of breast cancer ranging from 28 to 60% were observed (Messina, 2010).

Investigations on mammary gland morphology and cell differentiation were carried out in animal models to understand how isoflavone exerts the described protective effects. The results indicated that isoflavones might have been exerting its chemoprotective effect by stimulating early cell differentiation leading to a reduction in the number of least differentiated structures in the breast tissue (TEBs) which are susceptible to chemical carcinogens (Bouker et al., 2000; Lamartiniere, 2000).

Therefore, animal and epidemiological studies are consistent with the hypothesis that childhood and/or adolescence is the critical period for isoflavone of exposure (Messina et al., 2006) and also corroborates with speculations that Asian low breast cancer rates is derived from early exposure to soy products, including during pregnancy (Lamartiniere, 2000).

4.2.6 Variables influencing inconsistent outcomes

To better interpret and understand data about isoflavone intake and breast cancer it is important to consider the strengths and weaknesses of a wide variety of experimental models and designs (Messina et al., 2006). As commented previously, the major sources of limitation in those studies are the inappropriate experimental designs and incomplete or unclear information provided about food isoflavone content, patient description, serum

isoflavone levels, time of exposition, and isoflavone metabolism. There are studies in which some variables are not even considered. Differences in these factors can considerably difficult comparisons among studies generating misinterpretations of data and are probably related to the majority of inconsistent results in the literature.

Epidemiologic studies should take into consideration that the amounts of isoflavones are not equal in all soy foods. Indeed, there are notably differences in soy food preparations and isoflavone content. Raw soybeans contain nearly 1.0 mg/g (range of 0.4 –2.4 mg/g of total isoflavones while traditional soy foods (i.e., tofu, miso, natto) typically contain 0.2– 0.4 mg/g (Messina et al., 2001). In addition, the content and structure of isoflavones are altered when soy food undergo processing, which was shown to potentially affect the effects on breast cancer (Murphy et al., 1999; Allred et al., 2004).

Moreover, soy isoflavone content can vary according to local, weather, seed maturation, and breeding conditions. It was observed that during the process of seed maturation the contents of isoflavones decrease, whereas sprouting led to a continuing increase of isoflavone content. Interestingly, the protein extracts from the developing seeds showed clearly opposite effects on cell viability and inhibition of foci formation compared with those from sprouting seeds (Park et al., 2005).

Individual differences in the absorption and metabolism of ingested isoflavones are another variable not usually addressed on breast cancer studies. To evaluate the potential risks and benefits of phytochemicals to human health, it is important to know the physiological behavior of these compounds after ingestion (Hsieh et al., 2010). As previously commented, isoflavones are metabolized by intestinal microorganisms, which may be heterogeneous among individuals. This variability may have large contributing effects on the serum levels of free isoflavones and correspondent metabolites and thereby on the resulting physiologic effects. Furthermore, differences in isoflavone metabolism and bioavaiability should also be considered when analyzing data from rodent animal models since a higher percentage of both genistein and daidzein appear in the free or glycone form in rats (Gu et al., 2006).

Individual variabilities should also be considered when analyzing the effects of isoflavone intake in estrogen and progesterone serum levels. Menstrual cycle length varies significantly among women and analysis of reproductive hormone levels in single periods may not provide accurate data on isoflavone effects. In this case, it would be more appropriate to measure the hormone levels during the whole menstrual cycle (Lu et al., 2000). Different times of isoflavone exposition are yet another type of limitation that influences data interpretation. For example, short periods of exposition (such as 2 weeks) may provide data regarding only the acute effects of soy isoflavone intake on breast cancer, thus, limiting comparisons with long-term studies.

Other potential source of variability in clinical studies includes incomplete patient description. Different and more specific correlations would be obtained whether patients were also addressed in subgroups by ER status, serum estrogen levels, and type of treatment being received (e.g. tamoxifen) for example. Those detailed patient information should also be used as valuable adjustment parameters for raw data in order to improve interpretation accurateness (Shu et al., 2009; Messina et al., 2010).

Clearly, there is a need to encourage further detailed studies to reduce the heterogeneity of soy exposure data (Rochfort et al., 2007). Several recommendations have been made to improve study conditions and data interpretation such as: provide clear information about the isoflavone content (including glycone amount) on test products; include detailed description of products, concentrations, and amounts used; relate study conditions to usual

soy and isoflavone intakes and/or tissue levels of isoflavones; consider the risks and benefits of research findings for human health; and outline the benefits and limitations of the model system used when conducting cell culture or animal studies (Erdman et al., 2004).

5. Protease Inhibitors

5.1 Structure and bioavalability

Protease inhibitors (PIs) have been isolated from black-eyed peas (*Vigna unguiculata*), soy (*Glycine max*), brazilian pink bean (*Phaseolus vulgaris*), pea (*Pisum sativum*) and lentil (*Lens culinaris*) (Losso, 2008). In seeds, these molecules are involved in the regulation of endogenous proteases and in defense-related strategies against seed-eating insects and microorganisms (Ryan, 1990). The concentration of PIs is affected by the stage of seed development and sprouting. For example, soy-derived BBI content increases during the process of seed maturation while it decreases with soaking time during sprouting (Park et al., 2005).

PIs are classified in more than 20 families according to their inhibitory activity and structural features (Laskowski et al., 2000; Joanitti et al., 2006). The primary families found in legumes are the Kunitz and the Bowman-Birk which are involved in the inhibition of serine proteases (Joanitti et al., 2006; Losso, 2008). Kunitz PIs consist of proteins with molecular mass ranging from 6-20 kDa. These inhibitors are cross-linked by 2-3 disulfide bonds and have one reactive site that generally binds to trypsin. Bowman-Birk PIs are small proteins (6-15 kDa) presenting 5-7 disulfide bonds and 2 different and independent reactive sites located at opposite regions of the molecule (Fig. 2). Due to this double-headed configuration, these inhibitors can interact with 2 enzymes simultaneously (e.g. trypsin and chymotrypsin or trypsin and trypsin (Freitas et al., 1999; Ventura et al., 1966)). The disulfide bonds content of Kunitz and Bowman-Birk PIs are responsible for their remarkable structural stability (Joanitti et al., 2006). It has been reported that the inhibitory activity of these molecules is preserved after being exposed to a wide range of temperatures (up to 100ºC) and pHs (2-13) (Silva et al., 2001; Ho et al., 2008; Ye et al., 2009).

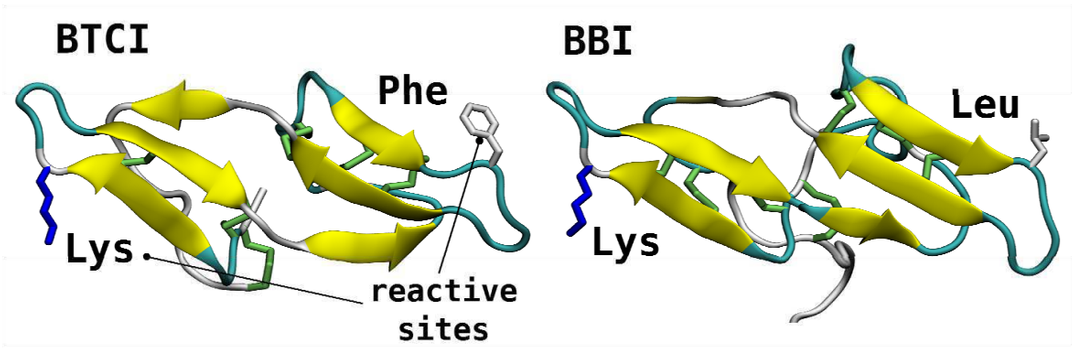

Fig. 2. Tridimensional structure of the Black-eyed pea trypsin and chymotrypsin inhibitor (BTCI) (Barbosa et al., 2007); PDB access number 2G81) and Bowman-Birk inhibitor (BBI) (PDB access number 1BBI) in ribbon representation. The disulfide bonds (in green) and reactive sites for trypsin (Lys) and chymotrypsin (Phe or Leu) are indicated. The image was made with VMD (Theoretical and Computational Biophysics Group, NIH Resource for Macromolecular Modeling and Bioinformatics, Beckman Institute, University of Illinois, Urbana-Champaign).

Because Kunitz and Bowman-Birk PIs are involved in the inhibition of serine proteases, these molecules have been considered as antinutritional factors able to impair digestion. Proper thermal seed processing have been described to eliminate the majority of legume antinutritional factors (Lajolo et al., 2002); nevertheless, some Kunitz and Bowman-Birk PIs are able to resist both acidic conditions and the action of proteolytic enzymes and pass through the stomach and small intestine without major degradation (Clemente et al., 2010). The metabolism and absorption of a soy Bowman-Birk PI (termed BBI) have been well characterized following oral administrations. After ingestion, BBI are rapidly metabolized and absorbed. BBI can resist stomach and small intestine conditions permitting the reach of significant amounts to the large intestine in the active form to exert their reported anticancer and anti-inflammatory properties (Kennedy, 1998). This PI is widely distributed throughout the body including breast tissue (Kennedy, 1998). In mice, BBI metabolites can be found in the liver, serum, and kidneys of mice 1.5 hours after ingestion (Wang et al., 2000). In humans, BBI excretion rates reach the peak within 6 hours and decrease to baseline levels within 12-24 hours (Wan et al., 2000).

PIs have been viewed as toxic agents inhibiting the growth of young animals and, perhaps, contributing to the development of pancreatic cancer. However, the effect on the promotion of atypical growth in rat pancreata, is not expected to occur in humans (Kennedy, 1998). Despite preserving their biological activity after passing through the gastrointestinal tract, it seems that these inhibitors do not act as antinutritional factors since they are not implicated in significant side effects even when ingested in concentrations far higher than the therapeutic dose (Kennedy, 1998).

5.2 Effects on breast cancer

PIs have been considered promising compounds in several economic and clinical areas. These inhibitors are multiple functional molecules with properties varying from insecticide to therapeutical activity in fields as immune systems, microorganism and viral infections, hemostasis, and cancer (Joanitti et al., 2006). PIs have shown promising anticancer effects on the prevention and suppression of cancer in several organ systems and tissue types (e.g. colon, liver, lung, esophagus, oral epithelium, ovarian, prostate, hematopoietic cells, and connective tissue), *in vitro* and *in vivo* (Kennedy, 1998). Particularly, BBI (Bowman-Birk Inhibitor from soy) was recognized as an investigational new drug by the Food and Drug Administration (FDA) and is currently being evaluated in clinical trials against pre-malignant oral cancer lesions, showing successful results on the reduction of cancer lesions with low or no side effects (Armstrong et al., 2000). Despite having their anticancer activity being widely investigated on different tumor types, few studies have addressed the effects of Kunitz and Bowman-Birk PIs specifically on breast cancer.

The effects of soy BBI on DMBA-induced transformation were investigated using an *in vitro* whole organ culture system of mouse mammary glands. It was observed that soy BBI and its palmitic acid conjugate (Pal-BBI) were effective in preventing DMBA-induced transformation, especially when added to the medium during the developing period after the exposure of mammary glands to DMBA, showing 35.9 and 53.4% prevention, respectively. Pal-BBI was also effective in decreasing the transformation incidence (32.2%) while BBI was not (10.3%) when only present in the medium before the promotion period. Possibly, the high lipophilicity of Pal-BBI increased its tissue retention and resulted in higher chemopreventive effects (Du et al., 2001).

An interesting *in vivo* study reported the effects of one year-long feeding of field bean meal (a rich source of PIs with 24% protein content), on mouse mammary tumor virus (MMTV)-induced mammary tumorigenesis in mice. The animals were fed chow with 2%, 4%, and 8% field bean protein (FBP) for 49 weeks and the incidence of mammary tumors was recorded in 58 weeks. A suppressive effect on mammary tumorigenesis was observed with increased FBP intake as mice fed with 2%, 4%, and 8% FBP showed significant tumor incidence reduction of 68%, 75% and 81% respectively. Adverse growth effects were observed only in mice receiving the 8% FBP-fed group (Fernandes et al., 1997).

In addition to chemopreventive properties, PI effects on the viability of breast cancer cells have also been reported. A Bowman-Birk PI isolated from Hokkaido large black soybeans seeds and a Kunitz PI isolated from Chinese black soybean seeds have been described to suppress the proliferation of breast cancer cells (MCF-7) *in vitro* (Ho et al., 2008; Ye et al., 2009). Similar effects were observed in MCF-7 cells treated with Bowman-Birk PIs derived from soy or black-eyed peas (Zhang et al., 1999; Joanitti et al., 2010). Conversely, it was described that soy BBI was not able to induce anticancer effects on ER-negative breast cancer cell MDA-MB 231 (Hsieh et al., 2010). It has been suggested that additional investigations should be made to determine whether tumor cell types and their specific carcinogenesis pathways may be determinants of the cancer chemopreventive properties of BBI (Hsieh et al., 2010).

PIs have been described to act on different cancer stages and activities impairing malignant cell transformation, altered gene expression and proteolytic activity, tumor growth, angiogenesis, and metastasis. These effects have been suggested to be linked to their protease inhibition activity (Kennedy, 1998; Joanitti et al., 2006). Nevertheless, the precise mechanisms by which PIs exerts their preventive and suppressive anticancer effects are not completely elucidated on breast cancer.

One of the main effects observed in tumor cells treated with PIs is a reduction on cell proliferation rates (Wan et al., 1998; Zhang et al., 1999; Chen et al., 2005). In 2005, Chen et al. reported a landmark research study revealing some clues about the mechanisms involved in this growth inhibition. They observed that the soy-derived BBI was able to inhibit proteasome activity, specifically the chymotrypsin-like domain, *in vitro* and *in vivo* in MCF-7 breast cancer cells. Proteasomes are large protein complexes acting in the degradation of misfolded proteins and regulation of particular proteins levels involved in intracellular pathways. Proteasome inhibition by BBI resulted in an accumulation of ubiquitinated proteins and proteasome substrates, such as cell cycle inhibitors p21Cip1/WAF1 and p27Kip1, inducing cell cycle arrest at G1/S phase. Furthermore, an up-regulation of MAP kinase phosphatase-1 (MKP-1) accompanied by a decrease of phosphorylated extracellular signal-related kinases (ERK1/2), which is a pathway involved in cell division, was also observed (Chen et al., 2005).

In addition to tumor growth inhibition, the BBI found in black-eyed peas (termed BTCI) was also shown to induce death on MCF-7 cells (Joanitti et al., 2010). The treatment of cells with BTCI induced significant reduction of the cell proliferation (arrest in S and G2/M phase) accompanied by significative DNA fragmentation, mitochondrial swelling, morphologic alterations, annexin-V+ cell number increase, and mitochondrial membrane potential reduction. These cytotoxic effects at first suggested that BTCI induced apoptosis cell death on MCF-7 cell. However, other features observed such as large lysosomes presenting weak acidification pattern followed by an increase in cytoplasmic acidification indicated another cell death pathway related to lysosomes: the lysosomal membrane permeabilization (LMP).

LMP is characterized by a perturbation of lysosomal membrane function leading to the translocation of lysosomal hydrolases from the lysosomal lumen to the cell cytoplasm. Therefore, the authors suggested that BTCI was able to induce both LMP and apoptosis processes on breast cancer cells (Joanitti et al., 2010).

The ability to induce tumor growth inhibiton and or cell death might be determined by PIs structural features, especially on the reactive sites, leading to different affinities to the targets. Moreover, variations in the dose and time of exposure are also important factors to be considered. For example, low Bowman–Birk PIs concentrations (10–40 μM) and long incubation periods (6–14 days) are frequently associated with proliferation inhibition (Wan et al., 1998; Zhang et al., 1999); while high concentration (200 μM) and short incubation periods (3 days) has been described to induce both tumor growth inhibition and cell death (Joanitti et al., 2010). Overall, these findings indicate that PIs are promising anticancer molecules and encourage more studies of these compounds on breast cancer.

6. Peptides

6.1 Structure and bioavailability

Among bioactive peptides found in legumes, a peptide isolated from soybean cotyledon has stand out as a potential anticancer agent. Lunasin is a 43-amino acid peptide with structurally conserved helix region containing Arg- Gly-Asp (RGD) cell adhesion motif followed by 9 aspartic acid residues at the carboxyl end. This peptide exhibits the primary sequence, SKWQHQQDSCRKQLQGVNLTPCEKHIMEKIQGRGDDDDDDDDD (Hernandez-Ledesma et al., 2009).

Lunasin has been identified in several soybean varieties with concentrations ranging from 4.4 to 70.5 mg lunasin/g protein (Gonzalez de Mejia et al., 2004; Hernandez-Ledesma et al., 2008). These concentrations are affected by the stage of seed development and sprouting. A notable increase of lunasin content is observed during seed maturation while the opposite occurs during sprouting. Breeding conditions (light and dark cycles) do not seem to affect the content of this peptide (Park et al., 2005). Large-scale processing of soy also influences lunasin concentration which was observed to vary from 12 to 44 mg lunasin/g of flour among different U.S. commercially available soy proteins (Gonzalez de Mejia et al., 2004).

Besides presenting heat stability, *in vitro* digestibility studies have shown that lunasin is digested by pancreatin (Galvez et al. 2001). However, animal studies indicate that when lunasin is ingested in combination with soy protein extract, it survives digestion and about 35% of the total oral dose is absorbed within 3 hours (de Lumen, 2005). These findings suggest that other components of soy are protecting lunasin from degradation (see section 7). Lunasin is biodistributed in various tissues including lung, liver, mammary gland, prostate and even the brain within 6 hours after administration. In addition, analysis of the liver and blood showed that this peptide was present in an intact and bioactive form (Jeong et al., 2007; Hsieh et al., 2010).

6.2 Effects on breast cancer

Lunasin was first discovered during studies regarding soy seed development. Early soybean seed development is characterized by orchestrated events of rapid cell division and differentiation. It was observed that the stage of seed cell expansion (massive synthesis of storage molecules) began after cell division had ceased and that a temporal production of lunasin coincided with this mitotic arrest. This data led to the hypothesis that lunasin could

also be involved in the disruption of mammalian cell division such as cancer cells. Indeed, lunasin was shown to block mitosis in mammalian cells by binding to chromatin and impairing the formation of the kinetochore complex in the centromere. These effects lead to the failure of microtubules to attach the centromeres and thereby to mitotic arrest and cell death (Galvez et al., 1999).

The mechanism of action for lunasin in the prevention of cell malignant transformation is related to chromatin binding. The dynamics of histone acetylation and deacetylation in non-cancerous cells is involved in chromatin remodelling which is implicated in cell cycle control (Hernandez-Ledesma et al., 2009). These processes are tightly regulated by tumor suppressor molecules which have among other activities the function to keep the histone core in a deacetylated (repressed) state. Nevertheless, during cell malignant transformation, many tumor suppressor molecules are inactivated by chemical and viral carcinogens which lead to the exposure of histones core. At this stage, lunasin is able to inhibit histone acetylation by binding deacetylated histones which prevents transcription and represses cell cycle progression (Galvez et al., 1999; Hernandez-Ledesma et al., 2009). In this context, lunasin can act as a surrogate tumor suppressor. Therefore, it has been suggested that lunasin selectively kills cells that are being transformed by disrupting the dynamics of histone acetylation-deacetylation when a transforming event occurs (Hernandez-Ledesma et al., 2008).

The RGD motif also contributes to the anticancer effects of lunasin. Since RGD motif is implicated in the attachment of tumor cells to the extracellular matrix, peptides containing this motif have been described to prevent metastasis of tumor cells by competitive adhesion to the extracellular matrix. Furthermore, it has been suggested that the internalization of lunasin in MCF-7 cells would be mediated by a functional RGD motif (Galvez et al., 1999).

In *in vitro* studies, lunasin was shown to suppress colony formation induced by the ras-oncogene in MCF-7 cells (Jeong et al., 2003). The *in vivo* effects of lunasin were investigated on an ER-negative MDA-MB-231 breast cancer model in which athymic mice received intraperitoneal injections of lunasin (4 or 20 mg/kg body weight) for 2 months prior to tumor implantation. After 7 weeks, mice treated with lunasin showed a significant reduction in breast tumor incidence and a delay in the appearance of tumors. In addition, histologic analysis revealed low proliferation and high apoptosis indexes in tumors of lunasin-treated mice (Hsieh et al., 2010).

7. Combined effects of bioactive compounds

The combination of therapies has emerged as an interesting approach for cancer prevention and treatment. This alternative strategy is based on synergistic effects of 2 or more anticancer compounds able to act in multiple targets resulting in a more efficacious treatment. Moreover, the combination of agents can result in significant activity at concentrations where the single agent is inactive. Thus, there is possibility to regulate an optimal dose and reduce unwanted side-effects (Lane, 2006).

As extensively commented here, legumes are rich sources of anticancer compounds including not only isoflavones, protease inhibitors and peptides but also saponins, phytic acid, and inositol phosphates. These molecules have different mechanisms of action on cancer cells, which suggests that their combination would result in synergistic effects. For example, a soy extract containing isoflavones and saponins significantly reduced the incidence of mammary tumour induced by DMBA (Gallo et al., 2001; Jin et al., 2002).

Inhibition of both breast tumor growth and metastasis was observed in animals treated with isolated soy proteins and isoflavones (Yan et al., 2002). Supplementation of isoflavone-containing crude soy protein to a transgenic mouse model for mmammary tumor significantly prolonged the latency period of tumour development (Jin et al., 2002).

The effects of combining legume bioactive compounds with other dietary molecules have also been studied. The combination of soy phytochemicals at a low dietary level with tea showed synergistic effects on inhibiting the growth of MCF-7 tumours (Zhou et al., 2004). Velie et al. undertook a large diet-based cohort study (40,559 postmenopausal women) and found that the only diet with significant negative correlation with invasive breast cancer was the traditional southern diet, which comprises high legume intake, low mayonnaise intake, and potentially cabbage intake (Velie et al., 2005).

The potential synergisms among legume bioactive compounds provide a clue to explain the different effects observed between studying the bioactive compound alone and evaluating a specific bioactive compound often associated with other anticancer molecules present in the legume. Therefore, researchers should consider the characterization of each legume extract or food not only in terms of quantity of the studied molecule but also in terms of identifying the presence of other potential bioactive compounds. This approach should improve data quality and allow more reliable comparisons and conclusions regarding the benefits and risks of legume intake.

In addition, the elucidation of these synergistic mechanisms may be useful to clarify the real effect of each bioactive compound and, based on this knowledge, aid in the design of novel preventive/therapeutic approaches and dietary guidelines. Chiesa et al. compared the breast tumor development of mice receiving a diet with different concentrations of isoflavones and soy protein and concluded that only animals receiving an isoflavone-poor soy protein concentrate diet showed reduction in tumor progression rates and metastasis development (Chiesa et al., 2008). Another study aimed to elucidate the synergistic effects between the major bioactive components present in a soy extract termed "Bowman-Birk Inhibitor Concentrate" (BBIC). BBIC was developed for use in large-scale human cancer-prevention trials and has been extensively studied for its bioactivity against several cancer types (Kennedy, 1998). Its anticancer effects have been mainly attributed to the soy-derived BBI, which is present in high concentrations in this extract (Kennedy, 1998). In addition to BBI, lunasin peptide is also present and both represent 44% of total protein in BBIC. BBI and lunasin peptide were administered intraperitoneally and separately in mice for 2 months prior to the implantation of MDA-MB-231 breast cancer cells. Surprisingly, it was observed that only lunasin was effective in inducing significant chemopreventive and therapeutic effects. In this context, BBI and other PIs present in BBIC complement lunasin activity in other manner. Since lunasin is easily degraded by gastrointestinal enzymes when ingested in a pure form, it has been reported that those PIs protect lunasin from digestion and make this peptide bioavailable (Hsieh et al., 2010).

Approaches of combining legumes bioactives compounds with conventional chemotherapeutic drugs have been shown promising results. Ito et al. reported that the combination of 10% miso diet (soy food) with 2.5 mg/kg tamoxifen resulted in a significant reduction in the incidence and multiplicity of MNU-induced mammary carcinomas mice (Ito et al., 1996). The combination of tamoxifen and soy protein isolate provided a better protection than using the components alone. The tumor incidence in DMBA-induced mammary carcinogenesis mice was reduced 29% by tamoxifen, 37% by soy protein isolate and 62% by the combination (Constantinou et al., 2001).

The high intake of soy isoflavones was associated with reduced risk of ER-positive breast cancer recurrence in patients receiving anastrozole treatment (aromatase inhibitor). In addition, it was suggested that the observed effect might be related to a synergistic inhibitory effect of isoflavones and anastrozole on the synthesis of estrogen (Kang et al., 2010). Another interesting combination with centchroman (selective estrogen receptor modulator) and soy intake was investigated on DMBA-induced mammary carcinogenesis. The doses and periods of treatment were optimized and a maximum tumor regression of 98.6% were achieved with centchroman 5 mg/kg per day, alone/in combination with soy 3×10^4 mg/kg per day at 5 weeks treatment period (Mishra et al., 2010).

8. Conclusion

Undoubtedly, plant species are notable sources of compounds essential to human health not only in terms of nutrition but also in a therapeutic aspect. In particular, the variety of legume-derived bioactive compounds has attracted the attention of researchers for their health-promoting properties on breast cancer. Encouraging results have identified the preventive and treatment effects of these compounds on breast cancer in *in vitro*, *in vivo* and epidemiological studies. However, other data sets indicate harmful or neutral outcomes. Therefore, the discussed data do not allow conclusive statements regarding the effects of legumes and their bioactive compounds on humans, especially on women at high risk for breast cancer and breast cancer patients.

Re-evaluation of current data and further studies are crucial to elucidate such doubts. Nevertheless, in order to provide expressive contributions and to make studies comparable, the design and interpretation of new experiments should be considered. In this context, the influences of variables, such as dose and time of exposition, and potential synergisms or antagonisms among compounds need to be investigated. Indeed, further studies on the effects and mechanisms of action of these molecules on breast cancer will provide a better comprehension regarding the safety of legume intake and the elaboration of suitable dietary guidelines.

The conclusive characterization of legumes as preventive and therapeutic anticancer compounds would lead to interesting future perspectives. Among other options, it would be possible to develop therapies based on legumes compounds; to improve their bioavailability by the use of drug delivery systems; to use them as templates in the process of rational design of new anticancer drugs; and even to control the amounts of specific bioactive compounds produced by genetically engineered legume crops.

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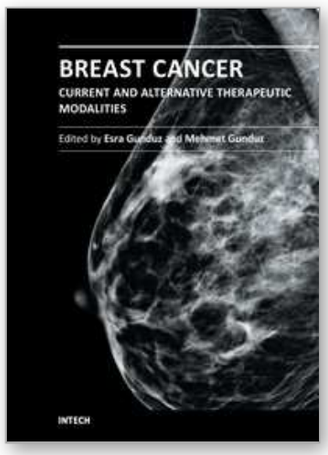

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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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