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Lunasin, a New Breast Cancer Chemopreventive Seed Peptide

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1. Introduction

With a prevalence of about 4.4 million women and a lethality rate of more than 410,000 cases per year, breast cancer is the most common cancer disease and the leading cause of death in women worldwide (Mangiapane et al., 2008). Advances in early detection and improved treatment for breast cancer have led to a steady decrease in the overall breast cancer mortality rate. However, breast cancer remains a significant cause of morbidity and mortality. In general, breast cancer is categorized into the estrogen receptor (ER)–positive type and the ER-negative type, based on the prevalence of ERs within the cell. About 70-80% of all breast cancers are estrogen sensitive and they are treated by conventional procedures including surgery, radiation chemotherapy, and estrogen analogues. Results from clinical trials have demonstrated that it is possible to prevent estrogen-responsive breast cancers by targeting the ER with selective modulators (i.e. tamoxifen, raloxifene, or lasofoxifene) or with aromatase inhibitors (i.e. anastrozole, letrozole, or exemestene). While the ER-positive tumors respond to anti-estrogen therapy and have better prognosis, the ER-negative tumors are more aggressive and resistant to treatments (Uray & Brown, 2006; Li & Brown, 2007; Cuzick, 2008). The high aggressiveness and therapeutic resistance of ER-negative breast tumors have made necessary the development of new and efficient strategies for their prevention and/or treatment.

Recently, the World Cancer Research Fund/American Institute for Cancer Research has reported that changes in diet and lifestyle are good strategies for cancer prevention. Evidence based on a systematic review of the published literature has demonstrated benefits of diet modification approach to reduce cancer risk (Greenwald & Dunn, 2009). The rising prevalence of cancer worldwide and the corresponding rise in health care costs is propelling interest among researchers and consumers for multiple health benefits of food compounds, including reduction in cancer risk and modification of tumor behavior (Béliveau, 2007; Kaefer and Milner, 2008). A large number of natural compounds present in the diet have been demonstrated to lower breast cancer risk and sensitize tumor cells to anti-cancer therapies (Kaefer & Milner, 2008; Ramos, 2008; Hauner & Hauner, 2010). Among these dietary compounds, those present in plant foods and collectively termed phytochemicals,

have been identified as the most promising chemopreventive agents. They may affect different targets of the signal transduction pathways that modulate gene expression, cell cycle progression, proliferation, metabolism and/or apoptosis (Ramos, 2008; van Breda et al., 2008). High priority should be given to research aimed at the study of natural compounds that could potentially prevent the development of breast cancer in susceptible patients.

1.1 Role of dietary compounds against estrogen-receptor negative breast cancer

It is well-known that endocrine interventions are not an effective strategy to reduce the risk of ER-negative breast cancers (Uray & Brown, 2006; Li & Brown, 2007; Cuzick, 2008). Thus, in the last few years, the searching and development of chemopreventive agents against ER-negative breast cancer are attracting more attention. Recently, a number of novel chemopreventive agents targeting non-endocrine pathways have been developed and their capacity to prevent ER-negative mammary tumorigenesis has been demonstrated (Li & Brown, 2007). These agents include retinoids, cyclooxygenase-2 inhibitors, tyrosine kinase inhibitors, and growth factors. Further studies have shown the other compounds possess critical role on cell growth of ER-negative breast cancer, such as retinoid X receptors, vitamin D receptors, peroxisome proliferator-activated receptors, n-3 polyunsaturated fatty acids (PUFA) and several phytochemicals (Simeone & Tari, 2004; Uray & Brown, 2006; Spencer et al., 2009). This chapter is focused on those dietary compounds that have demonstrated a promising potential as chemopreventive agents against ER-negative breast cancer. These compounds, including vitamins, lipids, phytochemicals, proteins and bioactive peptides, have been reported to provide important protection against initiation, promotion or progression of breast cancer. Effects of these compounds as well as their mechanism of action are summarized in this chapter, as shown in Table 1. Moreover, special attention is given to peptide lunasin, identified in soybean and other plants, and which chemopreventive properties against breast cancer have been recently demonstrated.

1.1.1 Vitamins

Retinoids is a family of compounds that include both natural and synthetic derivatives of vitamin A or retinol. They have been found to play important regulatory roles in cellular proliferation, development, metabolism and differentiation (Gudas et al., 1994). It has been reported that these compounds prevent carcinogenesis in a variety of tissues including the breast (Yang et al., 1999). *All-trans*-retinoic acids (ATRA), the oxidized form of retinol, inhibit the proliferation of breast cancer cells predominantly by blocking the transition from G1 to S phase, and activating proteasome function, caspase cleavage and apoptosis (Toma et al., 1997; Son et al., 2007). The main mechanisms of 4-haptoglobin-related protein's apoptotic action have been associated with nitric oxide and reactive oxygen species production, ceramide function, and mitochondrial permeability transition (Simeone & Tari, 2004). Recently, it has shown a rapid decrease of histone H3 acetylation at position Lys 9 at the human telomerase reverse transcriptase promoter. It could be an important mechanism by which ATRA shuts down telomerase activity, thus mediating its antitumor effects in ER-negative breast cancer cells (Phipps et al., 2009). Unfortunately, their side effects, including hyperlipidaemia, muco-cutaneous and liver toxicity, have limited their extensive use in humans (Lee et al., 1993). Therefore, there is a growing body of studies developing novel synthetic retinoids or combination therapies to decrease their toxicity and increase their

Dietary compound	Cell line/Animal model	Chemopreventive activity	Reference
Vitamin A	- MDA-MB-231 and MCF-7 cells	- ↓ Cell proliferation, blocking of G1 to S phase transition	Toma et al.,1997
	- SKBR-3 cells	- ↓ Cell proliferation, G1 phase arrest; ↓ PKCα expression; ↓ ERK/MAPK phosphorylation, and RB dephosphorylation	Nakagawa et al., 2003
	- SKBR-3 cells	- Activates proteasome function, caspase cleavage, and apoptosis	Son et al., 2007
	- SKBR-3 and MCF-7 cells	- ↓ Telomerase activity, histone H3-lysine 9 acetylation inhibition, and ↑ apoptosis	Phipps et al., 2009
Vitamin D	- SUM-159PT cells	- ↑ Apoptosis associated with an enrichment of membrane bound bax, a redistribution of cytochome c from the mitochondria to the cytosol and PARP cleavage; ↓ cell invasion	Flanagan et al., 2003
	- MDA-MB-231 and MCF-7 cells	- ↓ RelB/RELB gene expression and pro-survival targets Survivin, MnSOD and Bcl-2 levels; ↑ sensitivity to gamma-irradiation, and ↑ MAP-kinases ERK1 and ERK2 activity	Mineva et al., 2009; Cordes et al., 2006
	- Xenograft MDA-MB-231-TxSA cells in a murine model	- Vitamin D deficiency promotes the tumor growth	Ooi et al., 2010
n-3 PUFAs	- MDA-MB-231 cells	- ↓ Surface expression of CXCR4 and attenuate the migration/invasion	Altenburg and Siddiqui, 2009
	- MDA-MB-231 cells	- ↓ Cell proliferation, ↑ apoptosis via a transient ↑ caspase-3 activity, the promotion of nuclear condensation, and ↓ invasive potential	Blanckaert et al., 2010
	- Xenograft MDA-MB-435 cells in nude mice	- ↓ Cancer growth and metastasis, ↓ insulin-like growth factor I and epidermal growth factor receptor expression	Chen et al., 2002
	- Xengraft MDA-MB-231 cells metastasis to bone in nude mice	- Prevents the formation of osteolytic lesions in bone, indicating suppression of cancer cell metastasis to bone through ↓ CD44 expression in mRNA and protein levels	Mandal et al., 2010
MUFAs	-Carcinogen-induced mammary tumor in rats	- Lower degree of morphological malignancy in mammary tumor	Costa et al., 2002
	- *In vitro* and *in vivo* laboratory studies	- Influences on stages of carcinogenesis, modification on cell membranes, actions on signal transduction pathways, effects on gene expression and protein activity	Escrich et al., 2006, 2007
Olive oil phenols	- *In vitro* and *in vivo* laboratory studies	- Protect DNA from oxidative damage, inhibit carcinogen activation, and activate carcinogen-detoxifying system	Escrich et al., 2006, 2007
Flavonoids	- MDA-MB 231 and MCF-7 cells	- ↑ Cell apoptosis and maintenance of genome stability through ↑ BRCA1 and BRCA2 gene expression and modulate the different genes, such as BAX, RB1, BRIP, p53	Caëtano et al., 2006; Bernard-Gallon et al., 2010
	- *In vitro* laboratory studies	- Modulation of epigenetic events, such as DNA methylation and/or histone acetylation	Banerjee et al., 2008; Gaur & Bhatia, 2009
	- HS578T, MDA-MB-231, and MCF-7 cells	- ↑ Cell apoptosis through a caspase-3-dependent pathway; arrest cell cycle in the G2/M phase	Lin et al., 2009
	- MDA-MB-231 cells	- ↑ Anti-invasion potency and MMP-3 inhibitory activity	Phromnoi et al., 2009

Table 1. Summary of the potential mechanisms of dietary compounds against ER-negative breast cancer.

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Dietary compound	Cell line/Animal model	Chemopreventive activity	Reference
Green tea polyphenols	- MDA-MB-231 cells	- ↑ Cell apoptosis and ↓ invasion through beta-catenin and AKT signaling pathway modulation	Thangapazham et al., 2007a
	- Xenograft MDA-MB-231 cells in nude mice	- Arrest cell cycle at G1 phase by down regulation the expression of Cyclin D, Cyclin E, CDK 4, CDK1 and PCNA; delaying the tumor incidence as well as ↓ tumor burden	Thangapazham et al., 2007b
Indole-3-carbinol	- MDA-MB-468 cells	- ↑ Cell apoptosis mediated ↓ phosphorylation and activation of protein kinase B/Akt, and ↓ NF-κB DNA binding activity	Howells et al., 2002
BBI	-Carcinogen-induced mammary mouse tumor in culture system	- Prevents carcinogen-induced transformation	Du et al., 2001
Lactoferrin	- MDA-MB-231 cells	- ↓ Cell proliferation	Hurley et al, 1994
	- MDA-MB-231, HBL-100, and MCF-7 cells	- Arrest cell growth at G1 to S associated with ↓ CDk2 and cyclin E protein levels, ↓ CDk2 and CDk4 activity, and ↑ p21 expression to maintain pRb in a hypophosphorylated form	Damiens et al., 1999
	- HS578T and T47D cells	- ↓ Cell viability and cell migration, and ↑ cell apoptosis	Duarte et al., 2011
Lactoferricin	- MDA-MB-435 cells	- DNA fragmentation and morphological changes consistent with apoptosis	Furlong et al., 2006
Lectins	- MCF-7, T47D, HBL100 and BT 20 cells	- ↓ Cell proliferation	Valentiner et al., 2003
	- MDA-MB-231 cells	- ↑ Cell cytotoxity	Park et al., 2004
	- Xenograft MDA-MB-231 cells in SCID mice	- ↓ Cell proliferation, ↓ HER2 gene expression; ↑ mice survival and delayed tumor development	Lee-Huang et al., 2000
Lunasin	- MDA-MB-231 cells	- ↓ Cell proliferation; arrest cell cycle in S-phase, ↓ expression of CDC25A, Caspase 8, and Ets2, Myc, Erbb2, PIK3R1 and JUN signaling genes; ↓ expression of cyclins D1 and D3, CDK4 and CDK6 protein levels	Hsieh et al., 2010b; Hernández-Ledesma et al., 2011
	- DMBA-induced transformed NIH/3T3 cell	- ↓ Foci formation and cell proliferation	Hsieh et al., 2011b
	- DMBA-induced mammary cancer in SENCAR mice	- ↓ Tumor incidence	Hsieh et al., 2010c
	- Xenograft MDA-MB-231 cells in nude mice	- ↓ Tumor incidence, ↓ cell proliferation, induction of apoptosis	Hsieh et al., 2010a

efficacy against breast cancer (Gediya et al., 2008). Several clinical studies using retinoids as breast cancer chemopreventive agents are currently undergoing. Phase III clinical trials are showing that retinoids potently reduced incidence of second breast malignancies in premenopausal women (Bonanni et al., 2007).

1,25-Dihydroxyvitamin D3 (1,25(OH)2D3), the biologically active form of vitamin D, is not only a secosteroid hormone essential for bone and mineral homeostasis but also it is a compound exerting a number of biological functions. Epidemiological investigations indicate that higher level of vitamin D intake is inversely associated with breast cancer risk (Shin et al., 2002). In contrast, an epidemiological study carried out in 107 countries has demonstrated that deficiency in vitamin D increases the risk of breast cancer (Mohr et al., 2008). Recently, it has also been demonstrated that vitamin D deficiency promotes the growth of human breast cancer MDA-MB-231-TxSA cells in a murine model of malignant bone lesions (Ooi et al., 2010). Treatment of 1,25(OH)2D3 has been found to decrease RelB/RELB gene expression and pro-survival targets Survivin, MnSOD and Bcl-2 levels, and to stimulate the MAP-kinases ERK1 and ERK2 activity in both ER-positive MCF-7 cells and ER-negative MDA-MB-231 breast cancer cells (Mineva et al., 2009; Cordes et al., 2006). Vitamin D affects different pathways, such as cell cycle, apoptosis, hormone receptors, angiogenesis, and hypoxia, all of which are related to the breast cancer growth, progression and metastasis by mechanisms independent of estrogen signaling. Moreover, vitamin D may have synergistic, additive, or antagonistic effects when combined with other therapeutic agents against breast cancer (Flanagan et al., 2003; Bertone-Johnson, 2009). Well-designed clinical trials should be needed to further address whether vitamin D is likely to play an important role in reducing risk, mortality and recurrence of breast cancer.

1.1.2 Dietary lipids

Relationship between breast cancer and dietary lipids has been extensively studied. Studies in animal models and observations in humans have provided evidence that a high intake of n-6 PUFAs stimulate several stages in the development of mammary cancer, from an increase in oxidative DNA damage to effects on cell proliferation, free estrogen levels to hormonal catabolism (Bartsch et al., 1999). In contrast, fish oil-derived n-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), seem to prevent cancer by influencing the activity of enzymes and proteins related to intracellular signaling, and ultimately cell proliferation. Recently, it has been demonstrated that these n-3 PUFAs exert potent anti-inflammatory, anti-apoptotic, anti-proliferative and anti-angiogenic effects (Spencer et al., 2009). These authors have reported that these compounds act through regulation of several growth factors, cyclooxygenase 2 (COX-2), prostaglandin-E2 (PGE2), nitric oxide, nuclear factor kappa beta (NF-κB), matrix metalloproteinases (MMP) and beta-catenin. Mandal and co-workers (2010) used a xenograft metastasis MDA-MB-231 mouse model, demonstrating that fish oil diet prevents the formation of osteolytic lesions in bone by inhibiting pro-metastatic molecule CD44 expression both in mRNA and protein levels. It has been also shown that both DHA and EPA significantly reduce surface expression of CXCR4 and attenuate the migration/invasion of MDA-MB-231 cells *in vitro* (Altenburg and Siddiqui, 2009; Mandal et al., 2010). Recently, Blanckaert et al. (2010) reported that n-3 PUFAs have anti-proliferative effect, induce apoptosis via a transient increase in caspase-3 activity, promote nuclear condensation, and reduce the invasive potential of MDA-MB-231

cells. Flaxseed is the richest source of n-3 PUFA alpha-linolenic acid among the vegetable oils. Chen et al. (2002) have shown that nude mice fed flaxseed enriched diet inhibited the MDA-MB-435 human breast cancer growth and metastasis in a xenograft model, and this effect was partly due to its down-regulation of insulin-like growth factor I and epidermal growth factor receptor expression. These studies indicate that naturally occurring n-3 PUFAs are emerging because of their potential to increase efficacy to breast cancer treatment without having any additional side effects.

Abundant epidemiological studies have attributed a potential chemopreventive effect to extra-virgin olive oil, rich in antioxidants and monounsaturated fatty acids (MUFA) such as oleic acid, which is associated with low incidence and mortality rates from cardiovascular disease and some cancers, including breast cancer (Escrich et al., 2007). Interestingly, a negative modulatory role of a high-virgin olive oil diet in the appearance and progression of experimental breast cancer has been described (Escrich et al., 2007). Moreover, mammary tumors from animals fed this kind of diet not only showed a benign clinical behavior but also a lower degree of morphological malignancy compared with control and high n-6 fat diet (Costa et al., 2002; Escrich et al., 2006). Oleic acid as well as other minor components could contribute for the biological effects of olive oil on the distinct carcinogenesis stages through different molecular mechanisms of action.

1.1.3 Phytochemicls

The low incidence of breast cancer among Asians may be explained in part by dietary habits. Epidemiological and experimental studies have shown convincing evidence that people consuming phytoestrogens-rich diets have lower incidence and mortality of breast cancer (Messina & Flickinger, 2002). Asian diets are rich in soybean products containing different compounds found to provide important protection against initiation, promotion and/or progression of breast cancer (Messina et al., 2006). These include isoflavones, saponins, phenolic acids, phytosterols, protease inhibitors, and bioactive proteins and peptides, such as lectins and lunasin.

Genistein, daidzein and glycitein are the three major isoflavonoids, with a chemical structure similar to estrogens, found in soybean and soy products which properties have been extensively studied (Park and Surh, 2004). Genistein has been identified as the predominant isoflavone contained in soybean. Accumulating experiments have concluded that genistein functions as a promising carcinogenesis inhibitor through different molecular mechanisms of action (Banerjee et al., 2008). In addition to its estrogenic effects, genistein has been reported to possess anti-carcinogenic effect through an ER independent pathway, thus being beneficial for ER-negative breast cancer. Genistein possesses free radicals scavenging activity, inhibits the expression of stress-response related genes, and inhibits the growth of several cancer cell lines through the modulation of genes intimately related to the regulation of cell cycle and apoptosis (Vissac-Sabatier et al., 2003; Liao et al., 2004). Mutations of the onco-suppressor genes BRCA1 and BRCA2 are associated with a hereditary risk of breast cancer. It has been demonstrated that genistein or daidzein treatments up-regulate BRCA1 and BRCA2 gene expression and modulate the different genes involved these pathways, such as BAX, RB1, BRIP and p53 in both MCF-7 and MDA-MB-231 cells, suggested a potential chemopreventive effect in promoting apoptosis and maintenance of genome stability (Caëtano et al., 2006; Bernard-Gallon et al., 2010). Genistein also intervenes in several cellular transduction signaling pathways and involves in the

regulation of gene activity by modulating epigenetic events such as DNA methylation and/or histone acetylation (Banerjee et al., 2008; Gaur & Bhatia, 2009). *Puerariae radix* is a popular natural herb and a traditional food in Asia. Isoflavones contained in this plant induce cell apoptosis through a caspase-3-dependent pathway and mediate cell cycle arrest in the G2/M phase in HS578T, MDA-MB-231 and MCF-7 cell lines (Lin et al., 2009). Recently, it has demonstrated that the flavonols quercetin and kaempferol have higher anti-invasion potency and higher MMP-3 inhibitory activity than genistein, genistin and daidzein in the MDA-MB-231 cells (Phromnoi et al., 2009). Phytochemicals, ingested through soybean or other legumes, exerts anti-carcinogenic effects through pleiotropic molecular mechanisms of action on cell cycle, apoptosis, angiogenesis, invasion and metastasis. As an adjuvant therapy in many chronic diseases like cancer, its use is almost established due to less or no side effects.

Epidemiological studies have shown that consumption of green tea polyphenols (GTP) reduces the incidence and progression of breast cancer. An inverse association between the risk of breast cancer and the intake of green tea has also been reported in Asian Americans. The breast cancer progression is delayed in the Asian population that consumes green tea on regular basis (Wu et al., 2003). Green tea is an important source of antioxidants that may be useful for chemoprevention of cancer. It was demonstrated that treatments of GTP and its principal constituent epigallocatechin gallate (EGCG) significantly induce apoptosis and decrease invasion of MDA-MB-231 cells, through beta-catenin and AKT signaling pathway modulation (Thangapazham et al., 2007a). Moreover, both GTP and EGCG treatments had the ability to arrest the cell cycle at G1 phase by down-regulation the protein expression of cyclin D, cyclin E, cyclin-dependent kinases (CDK) 4, CDK 1 and PCNA. In an *in vivo* study, nude mice were inoculated with MDA-MB-231 cells and treated with GTP and EGCG, observing that these compounds were effective in delaying the tumor incidence as well as reducing the tumor burden compared to the control group (Thangapazham et al., 2007b). The tumor sections were also observed that GTP and EGCG induce apoptosis and inhibit cell proliferation by immunohistochemistry analysis. Similarly, indole-3-carbinol, abundant in cruciferous vegetables, induces apoptosis inhibiting phosphorylation and activation of protein kinase B/Akt, and decreasing NF-κB DNA binding activity in the tumor-derived ER-alpha-negative breast cell line MDA-MB-468 (Howells et al., 2002).

1.1.4 Food peptides

Natural peptides have attracted attention as drug candidates owing to their possession of certain key advantages over alternative chemotherapy molecules. In contrast to most small-molecule drugs, peptides have high affinity, strong specificity for targets and low toxicity. Moreover, peptides have good penetration of tissue due to the small size, thus attractive as alternative cell surface targeting agents for cancer therapy (Bhutia and Maiti, 2008). Proteins and peptides have become one group of nutraceuticals exerted biological functions that shows potential results in preventing the different stages of cancer including initiation, promotion and progression (de Mejia and Dia, 2010). Recently, there has been increased interest in the potential health benefits of different food proteins and peptides, including plant protease inhibitors, lactoferrin and lactoferricin, lectins and lunasin.

Protease inhibitors, found in plant tissues particularly legumes, act as possible protective agents against several types of cancer, such as breast cancer. The Bowman-Birk inhibitor (BBI) is a soybean-derived protease inhibitor with 71 amino acids that has been shown to be

an effective suppressor of carcinogenesis in both *in vitro* and *in vivo* assays (Losso, 2008). The role of BBI in carcinogenesis was evaluated either as a purified BBI or as BBI concentrate (BBIC). BBI is involved in inflammation processes, decreases the amount of oxidative damage, and suppresses carcinogenesis by affecting the amount of certain types of proteolytic activities or the expression of certain proto-oncogenes. BBIC achieved Investigational New Drug status from the FDA in 1992, and human trials are currently undergoing to evaluate its use as an anticarcinogenic agent for prostate and oral carcinomas (Armstrong et al., 2000, 2003; Malkowicz et al., 2001). Although BBI has a broad spectrum of cancer-protective activities, its effects on breast cancer remains limited. Treatment of BBI was effective in preventing 7,12-dimethylbenz[a]anthracene (DMBA)-induced transformation using mouse mammary glands in culture system (Du et al., 2001). However, no *in vivo* study reporting the effect of BBI as breast cancer preventive peptide has been published. Nevertheless, there are some *in vitro* studies showing that BBI decreased estrogen-dependent human breast cancer cell growth. These studies demonstrated that suppressed proliferation of MCF-7 cells through abating proteasome function that resulted in up-regulation of MAP kinase phosphatase-1, which turns to suppress ERK1/2 activity and induce apoptosis and lysosome membrane permeabilization (Zhang et al., 1999; Chen et al., 2005; Joanitti et al., 2010). Kunitz trypsin inhibitor (KTI) is another protease inhibitor originally isolated from soybean. The biological significance of this protein in carcinogenesis is mainly attributed to suppress invasion and metastasis of cancer cells (de Mejia and Dia, 2010). KTI isolated from seeds of Chinese black soybean, suppressed proliferation of MCF-7 cells and HepG2 hepatoma cells (Ye and Ng, 2009). However, there are few data about the role of protease inhibitors against ER-negative breast cancer.

Lactoferrin, a globular glycoprotein with a molecular mass of 80 kDa, is a multifunctional protein of the transferrin family that is widely represented in various secretory fluids, such as milk, saliva, tears, and nasal secretions (Lönnerdal, 2009). Early study showed that bovine and human lactoferrin had no effect on growth of MCF-7 cells and only a minimal inhibitory activity toward the MDA-MB-231 line was observed (Hurley et al., 1994). Lactoferrin induces growth arrest at the G1 to S transition, through decreasing protein levels of CDK2 and cyclin E. CDK2 and CDK4 kinase activities are also decreased and p21 expression is increased, maintaining the retinoblastoma protein (Rb) in a hypophosphorylated form in MDA-MB-231 cells and other epithelial cell lines such as HBL-100, MCF-7 and HT-29 (Damiens et al., 1999). Recently, Duarte et al. (2011) has also provided evidences that bovine lactoferrin decreases the cell viability and cell migration, and increases apoptosis in HS578T and T47D cells. Bovine lactoferricin is a cationic peptide produced by gastric-pepsin hydrolysis of bovine lactoferrin, with potent cytotoxic activity against cancer cells (Bellamy et al., 1992). Lactoferricin caused DNA fragmentation and morphological changes consistent with apoptosis in MDA-MB-435 cell cultures, but did not affect the viability of untransformed mammary epithelial cells (Furlong et al., 2006). Although the mechanisms of action are not fully known, the results gathered in this work suggest that lactoferricin interferes with some of the most important steps involved in cancer development.

Extensive studies have revealed that a number of lectins from plants can be used for prevention and/or treatment of cancer. Soy lectins are a significant group of biologically active glycoproteins that have been shown to possess cancer chemopreventive activity by *in vitro*, *in vivo* and human case studies (de Mejia et al., 2003). A sialic acid-specific lectin has been purified from the mushroom *Paecilomyces Japonica*, which exerts cell cytotoxic effects on

the human breast cancer MDA-MB-231, human stomach cancer SNU-1 and pancreas cancer AsPc-1 cells (Park et al., 2004). The suggested mechanisms of action for lectins include effects on membranes of tumor cells, reducing cell proliferation and inducing apoptosis, as well as effects on macrophages increasing their tumor-specific cytotoxicity. Another potential mechanism of action includes lectins's effects on the immune system by altering the production of various interleukins (de Mejia and Prisecaru, 2005). GAP31 (Gelonium protein of 31 kDa) and MAP30 (Momordica protein of 30 kDa) are glycoproteins isolated from the medicinal plants *Gelonium multiflorum* and *Momordica charantia*, respectively. Lee-Huang et al. (2000) conducted a study demonstrated the efficacy of GAP31 and MAP30 inhibiting MDA-MB-231 cells proliferation and expression of HER2 gene, and also increasing survival delayed tumor development in human breast cancer bearing SCID mice. Moreover, some dietary lectins can inhibit cell growth of human breast cancer MCF-7, T47D, HBL100 and BT 20 cells *in vitro*, suggesting a protective effect of these plant lectins for breast cancer (Valentiner et al., 2003). There is still much to learn about the effects of plant lectins on cancer risk. However, they are currently being used as therapeutics agents in cancer treatment studies and this area of research holds considerable potential.

2. Lunasin's discovery and beyond

Lunasin is a peptide composed of 43 amino acid residues with a MW of 5.5 kDa, which sequence is SKWQHQQDSCRKQKQGVNLTPCEKHIMEKIQGRGDDDDDDDDD. It was initially identified in the soybean cotyledon when a cDNA encoding for a post-translationally processed 2S-albumin (Gm2S-1) was cloned from mid-maturation soybean seed (Galvez et al., 1997). Gm2S-1 coded not only for the methionine-rich protein that was sought to promote the nutritional quality of soy protein but also for other three proteins, a signal peptide, a linker peptide, and a small subunit. This subunit was termed lunasin from the Tagalog word "lunas" for cure. Galvez and co-workers observed that transfection and constitutive expression of the lunasin gene into mammalian cells disrupted mitosis and induced chromosomal fragmentation and apoptosis (Galvez and de Lumen, 1999). The authors attributed lunasin's effects to its negatively charged poly-D carboxyl end that could bind to the highly basic histones found within the nucleosomes of condensed chromosomes, probably to regions that contain more positively charged, such as the hypoacetylated chromatin found in telomeres and centromeres. The displacement by lunasin of the kinetochore proteins normally bound to the centromeres could lead to the failure of spindle fiber attachment and eventually to mitotic arrest and cell death. In addition, lunasin contains the sequence RGD, a cell adhesion motif, that is responsible for the attachment of lunasin to extracellular matrix (Galvez and de Lumen, 1999) thereby allowing its internalization in mammalian cells within a few minutes and its localization in the nucleus in approximately 18 h (Lam et al., 2003). The tri-peptide RGD is the cell attachment site recognized by integrins present in extracellular matrix and cell surface proteins (Ruoslahti and Pierschbacher, 1986). Previous studies have shown the role of RGD peptides inducing apoptosis in different cell lines via a caspase-dependent mechanism (Matsuki et al., 2008; Anuhadra et al., 2000). This motif has been also found to cause cytotoxicity in established human cancer cell lines, including HL 60 (Anuhadra et al., 2000).

Lunasin has been identified in soybean and other beans, grains and herbal plants, such as wheat, barley, rye, amaranth, sunberry, wonderberry, bladder-cherry and jimson weed etc., at concentrations ranged from 0.013 to 8.1 mg lunasin/g of protein (Jeong et al., 2002; de

Mejia et al., 2004; Jeong et al., 2007a, 2007b, 2007c; Silva-Sanchez et al., 2008; Jeong et al., 2009; Jeong et al., 2010a). Lunasin's concentration in seeds and its products has been reported to depend on the genotype varieties, some environmental factors, such as temperature and soil moisture, and the processing conditions (de Mejia et al., 2004; Wang et al, 2008). The stages of seed development have also been found to affect lunasin's concentration, and thus, a notable increase of this concentration has been found to happen during seed maturation. However, the content of lunasin is continually decrease accompanied with the soaking time of sprouting, but is not affected by light and dark conditions (Park et al., 2005). Recently, it has been found that environmental factors, such as germination time and temperature has a significant influence on the composition and concentration of bioactive compounds in germinated soybean flour from the Brazilian soybean cultivars BRS 133 and BRS 258 (Paucar-Menacho et al., 2010b, c). These authors reported that protein concentration also affect the final distribution of nutrients and bioactive components in soybean, including lunasin (Paucar-Menacho et al., 2010a).

A first study has demonstrated lunasin's presence in US commercially available soy foods, including soy milk, infant formulas, tofu, bean curd, soybean cake, tempeh, and su-jae (Hernández-Ledesma et al., 2009a). Concentrations of this peptide in soy milk and other soybean products seem to be determined by the soybean variety and the process used during manufacturing, indicating that these two parameters can be used to control contents of these two peptides. Previously it had been demonstrated that large-scale processing of soy to produce different protein fractions influences lunasin concentration. This content varied from 12 to 44 mg lunasin/g of flour when different commercially available soy proteins were analyzed (de Mejia et al., 2004; Jeong et al., 2003).

2.1 Lunasin's bioavailability

Oral administration of an anti-carcinogenic agent has been recognized as a plausible and cost-effective approach to reduce cancer morbidity and mortality by inhibiting precancerous events before the occurrence of clinical disease (Prasain & Barnes, 2007). Since lunasin is a peptide, it is crucial to establish whether it, once orally ingested, survives digestion and gets absorbed, reaching the target tissues and organs in an intact and bioactive state. Lunasin's bioavailability has been demonstrated by both *in vitro* and *in vivo* studies. Preliminary bioavailability studies carried out in mice and rats fed lunasin-enriched soy protein have found that 35% of ingested lunasin reaches the target tissues and organs in an intact and active form (Jeong et al., 2007a, 2007b). Park and coworkers carried out *in vitro* studies demonstrating the role of proteases inhibitors, such as BBI and KTI, in protecting lunasin from digestion by gastrointestinal enzymes when soy protein was orally consumed (Park et al., 2007). This protection plays a major role in making lunasin bioavailable in soy protein. Recently, it has been demonstrated that lunasin extracted from the blood and liver of lunasin-enriched soy diet fed rats is bioactive and able to suppress foci formation as synthetic lunasin does (Hsieh et al., 2010a). Lunasin's bioavailability has been also reported in a human study. Dia and co-workers demonstrated 4.5% of lunasin ingested in the form of soy protein reached plasma of healthy volunteer men (Dia et al., 2009a). The capacity of lunasin to survive degradation by gastrointestinal and serum proteinases and peptidases reaching blood and other organs in a bioactive form, make lunasin as a perfect candidate to exert a potent *in vivo* cancer preventive activity. This fact supports future clinical trials to investigate the chemopreventive activities of lunasin.

2.2 Lunasin's chemopreventive properties against breast cancer

Lunasin's chemopreventive activities have been demonstrated by both *in vitro* and *in vivo* studies. *In vitro* studies have demonstrated that cancer preventive properties of this peptide in mammalian cells induced by chemical carcinogens and viral oncogenes. *In vivo* studies, lunasin's preventive properties have also been confirmed in both skin and a breast cancer mouse model induced by a chemical carcinogen. Recently, it has been demonstrated that lunasin exerts a promising role against breast cancer both by using MDA-MB-231 cell culture and a breast cancer xenograft mouse model. Lunasin's chemopreventive properties against ER-negative breast cancer and its possible mechanisms are described in this section.

2.2.1 *In vitro* lunasin's chemopreventive properties – Mechanism of action

Lunasin was considered as a "watchdog" agent that sits in the nucleus of the cells and effectively does nothing when there is no transformation event. When a transformation event occurs, lunasin is triggered into action (de Lumen, 2005). Interestingly, recent studies have revealed that lunasin also acts on well-established cancer cell lines. Up to one third on breast cancers that are initially ER-independent making tumors resistant to endocrine therapy during tumor progression (Im et al, 2008). Due to this emergence of hormone-resistance, it is necessary to search for alternative therapies. Lunasin has been demonstrated to inhibit cell proliferation in ER-negative breast cancer MDA-MB-231 cells in a dose-dependent manner, showing an IC_{50} value of 181 μM (Hsieh et al., 2010b). Studies carried out to establish a structure/activity relationship showed an IC_{50} value of 138 μM for the 21 amino acid sequence localized at the C-terminus of lunasin, thus being the main responsible for lunasin's inhibitory effect on breast cancer cells proliferation (Hernandez-Ledesma et al., 2011). Possible mechanisms of action have been recognized as responsible for lunasin's chemopreventive action against breast cancer (Figure 1). First studies demonstrated that lunasin inhibits histone acetylation that is considered as one of the most important epigenetic modifications acting on signal transduction pathways involved in cancer development (Dwarakanath et al., 2008; Dalvai and Bystricky, 2010). Early mistargeted and deregulated histone acetyltransferase (HATs) activities occurring in the common tumor types, such as breast cancer might determine the subsequent genetic changes leading to tumor development and progression (Gayther et al., 2000; Stearns et al., 2007). Because epigenetic changes may be reversible, they represent an active area for new drug investigation and are promising targets for cancer therapy. Therefore, accumulating studies focus on investigating and developing the HATs modulators either for mechanistic studies or for anticancer values.

When the cells are in the steady-state conditions, the core H3 and H4 histones are mostly deacetylated, as a repressed state. After cells treatment with lunasin and sodium butyrate, a known deacetylase inhibitor, the process of histone acetylation was found to be inhibited in C3H10T1/2 fibroblasts and MCF-7 breast cancer cells (Galvez et al., 2001; Jeong et al., 2003). Furthermore, lunasin has demonstrated to compete with different HATs, such as yGCN5 and PCAF, inhibiting the acetylation and repressing the cell cycle progression (Jeong et al., 2002, 2007a, 2007b). According to these findings, an epigenetic mechanism of action for lunasin has been proposed. This model reveals that lunasin can selectively kill cells that are being transformed or are newly transformed when tumor suppressor proteins, like Rb, p53 and pp32, are inactivated by chemical carcinogens and/or viral oncogenes. When lunasin is present in the nucleus, it is acting as a surrogate tumor suppressor and tightly binding to

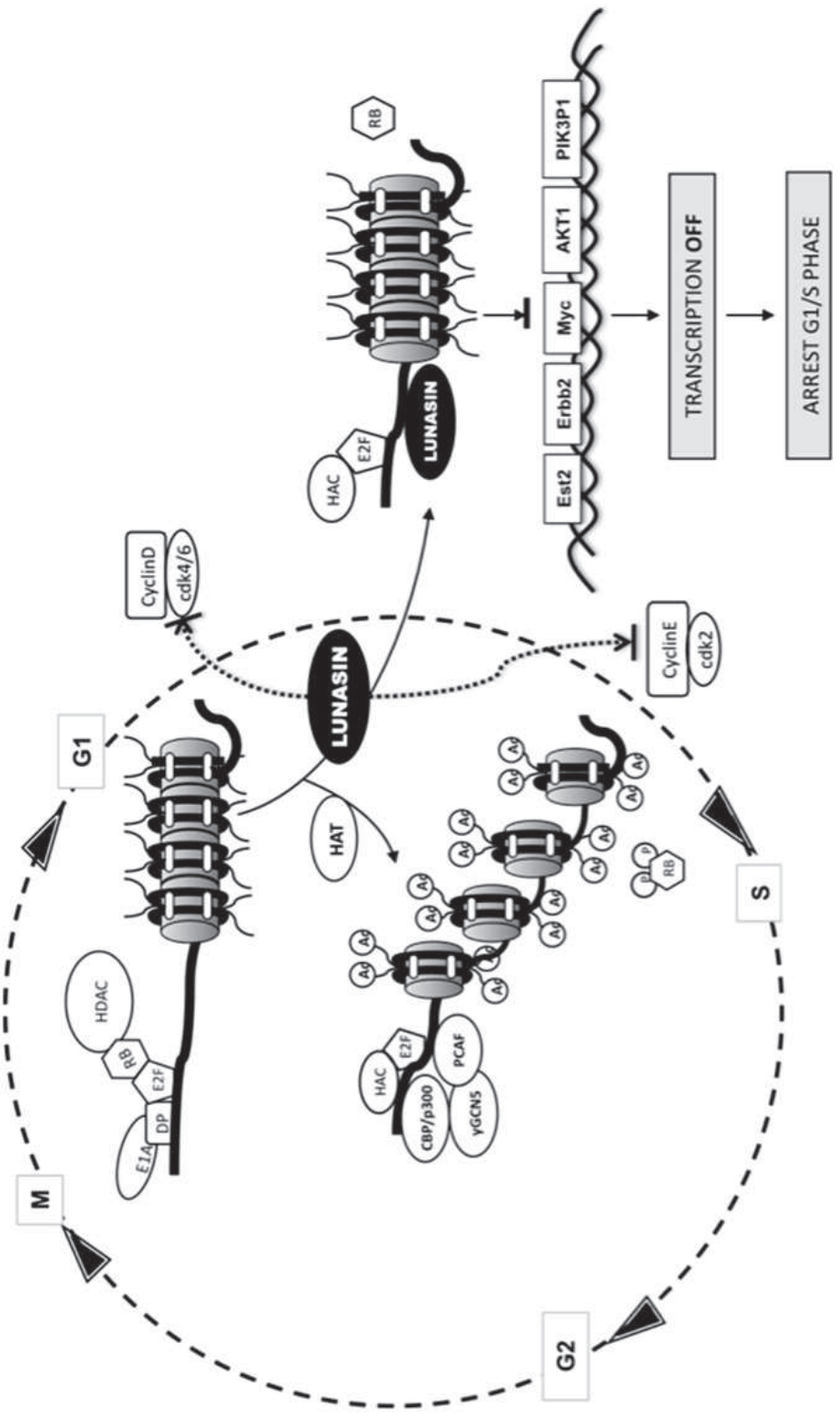
RB
LUNASIN
HAC
E2F
Est2
Erbb2
Myc
AKT1
PIK3P1
TRANSCRIPTION OFF
ARREST G1/S PHASE
CyclinD
cdk4/6
CyclinE
cdk2
LUNASIN
HAT
G1
S
G2
M
HDAC
RB
E2F
DP
E1A
HAC
E2F
PCAF
CBP/p300
yGCN5
Ac
P
RB

Fig. 1.

deacetylated core histones and disrupting the balance between acetylation-deacetylation, which is perceived by the cell as abnormal and leads to cell death (de Lumen, 2005). Recently, we have demonstrated that lunasin is a potent inhibitor of H3 and H4 histone acetylation (Hernández-Ledesma et al., 2011). This activity was higher than that demonstrated by other compounds, such as anacardic acid and curcumin, which chemopreventive properties have been demonstrated (Balasubramanyam et al., 2003, 2004a, 2004b). Studies focused on elucidating lunasin's structure-activity relationship establish that lunasin's sequence is essential for inhibiting H4 acetylation whereas poly-D sequence is the main active sequence responsible for H3 acetylation inhibition (Hernández-Ledesma et al., 2011).

Acetylation of specific lysine residues in histones is generally linked to chromatin disruption and the transcriptional activation of genes (Strahl & Allis, 2000). A plethora of chromatin alterations appears to be responsible for the development and progression of various types of cancers, including breast cancer. A global histone modification analysis revealed that in the majority of breast cancers, histone H4 acetylation at position Lys16 was reduced or absent, suggesting that this alteration may represent an early sign of breast cancer (Fraga et al., 2005). In addition, moderate to low levels of histone H3 acetylation at positions Lys9 and Lys18, and histone H4 acetylation at Lys12 were observed in breast carcinomas, and they were associated with poor prognosis and clinical outcome (Elsheikh et al., 2009). Therefore, evaluation of inhibitory effect on specific lysine residues of histones seems to be very promising for searching new therapies against breast cancer. A dose-dependent inhibitory effect on H4 acetylation at positions H4-Lys 8 and H4-Lys 12 was observed after addition of lunasin to breast cancer MDA-MB-231 cells, reaching 17% and 19% for both positions, respectively, when lunasin was treated at 75 μM (Hernández-Ledesma et al., 2011). It should be needed to extensively study the relevance of these results on lunasin's chemopreventive activity to provide data about lunasin's molecular mechanism of action on epigenetic alterations that would be very useful to define new prognostic markers and therapeutic targets.

Studies conducted in our laboratory have revealed different mechanisms of action than histone acetylation inhibition. We have demonstrated that lunasin modulates expression of different genes and proteins involved in cell cycle, apoptosis and signaling transduction (Hsieh et al., 2010b). Inhibition of deregulated cell cycle progress in cancer cells is being considered an effective strategy to delay or halt tumor growth. The cell cycle is regulated through the sequential activation and inactivation of CDKs that drive cell cycle progression (Kato et al., 1993). A pivotal regulatory pathway determining rates of cell cycle transition from G1 to S phase is the CCN/CDK/p16/RB pathway. Over-expression of cyclins D1 and D3 is one of the most frequent alterations present in breast tumors. Cyclins D interacts with CDK4 or CDK6 to form a catalytically active complex, which phosphorylates RB to free active E2F (Sutherland and Musgrove, 2004). Up-regulatory lunasin's effect of RB gene expression (Hsieh et al., 2010b), as well as its inhibitory effect of RB phosphorylation (Jeong et al., 2007b), suggest that both transcriptional and post-translational modifications may be responsible for lunasin's inhibitory effect on cancer cell cycle progression. Moreover, lunasin has been found to inhibit cell proliferation, arrest the cell cycle in the S phase in 45% and down-regulated the mRNA levels of CDk2, CDk4, CDC25A, Caspase 8, and Ets2, Myc, Erbb2, AKT1, PIK3R1 and Jun signaling genes in MDA-MB-231 cells (Hsieh et al., 2010b, 2011a). Lunasin was also demonstrated to reduce protein levels of cyclin D1, cyclin D3,

CDK4 and CDK6 in a dose-dependent form in these breast cancer cells (Hernández-Ledesma et al., 2011), that might also contribute on this lunasin's suppressive effect. This action also affects cell cycle control pathway, especially in the G1/S phase arrest. However, further research should be needed to elucidate the complete molecular and epigenetic lunasin's mechanism of action in breast cancer and other type of cancer cell lines.

Inflammation and oxidative stress are two of the most critical factors implicated in carcinogenesis and other degenerative disorders. Accumulating evidences have revealed that chronic inflammation is involved in the development of approximately 15–20% of malignancies worldwide (Kuper et al., 2000), being clearly associated with increased cancer risk and progression (Allavena et al, 2008). In the last years, there was an increasing body of evidence supporting the role of COX-2 in breast cancer development and progression. COX-2 has been found to be inappropriately induced and up-regulated in human breast cancer. Molecular studies have linked over-expression of COX-2 to a number of critical components of breast carcinogenesis including mutagenesis, angiogenesis, inhibition of apoptosis and aromatase-catalysed oestrogen biosynthesis. Moreover, high levels of COX-2 have been also associated with poor prognosis (Ristimäki et al., 2002; Singh-Ranger et al., 2008). Lunasin has been found to exert anti-inflammatory activity that might contribute to its chemopreventive properties against breast cancer. First studies demonstrated that lunasin potently inhibits lipopolysaccharide-induced production of pro-inflammatory mediators interleuquine-6, tumor necrosis factor-α, and PGE2 in macrophage cells (Hernández-Ledesma et al., 2009b), through modulation of COX-2/PGE2 and inducible nitric oxide synthase/nitric oxide pathways, and suppressing of NF-κB pathways (Dia et al., 2009b; de Mejia & Dia, 2009). Larkins and co-workers (2006) have demonstrated that COX-2 inhibition can decrease breast cancer cells motility, invasion and matrix metalloproteinase expression. Abnormally up-regulated COX and PGs expression are features in human breast tumors, so lunasin might have a role in treatment and prevention of this kind of cancer.

Large amounts of reactive oxygen species (ROS) have been shown to participate in the etiology of several human degenerative diseases, including inflammation, cardiovascular and neurodegenerative disorders, and cancer (Ames et al., 1993). Oxidative stress and ER-associated proliferative changes are suggested to play important roles in estrogen-induced breast carcinogenesis. Several transcription factors and tumor suppressors are involved during stress response such as Nrf2, NFκB and BRCA1 (Acharya et al., 2010). Physiologically achievable concentrations of estrogen or estrogen metabolites have been shown to induce ROS production. Estrogen-induced ROS by increasing genomic instability and by transducing signal through influencing redox sensitive transcription factors play important role in cell transformation, cell cycle, migration and invasion of the breast cancer (Okoh et al., 2011). Lunasin has also been found to exert potent antioxidant properties, reducing lipopolysaccharide-induced production of ROS by macrophage cells, and acting as a potent free radical scavenger (Hernández-Ledesma et al., 2009b). Recently, lunasin purified from *Solanum nigrum* L. has been found to protect DNA from oxidative damage by suppressing the generation of hydroxyl radical via blocking fenton reaction (Jeong et al., 2010b).

2.2.2 *In vivo* lunasin's chemopreventive properties

Lunasin's role as chemopreventive agent against breast cancer has also been demonstrated in *in vivo* mouse models. Our first findings show a relevant inhibitory effect on mammary

tumors development when a lunasin-enriched diet was administered to DMBA-induced SENCAR mice (Hsieh et al., 2010c). Six-week-old SENCAR mice were fed experimental diets before, during and after DMBA treatment by gavage once per week for 6 weeks, until they were sacrificed at 24-week-old. Tumor generation and tumor incidence were reduced by 38% and 25%, respectively, in the mice fed with lunasin-enriched diet (containing 0.23% lunasin) compared with control group. Moreover, the tumor sections obtained from mice fed the lunasin-enriched diet showed slight stromal invasion and degree of morphological aggressiveness due to the effect of this peptide contained in the soy protein preparation (Hsieh et al., 2010c). Park and co-works have reported that an isoflavone-deprived soy peptides prevent DMBA-induced rat mammary tumorigenesis, as well as inhibits the growth of human breast cancer MCF-7 cells in a dose-dependent manner, and induce cell death (Park et al., 2009). Lunasin might be responsible for the effects reported by these authors.

A recent study has demonstrated that lunasin reduces tumor incidence and generation in a xenograft mouse model using human breast cancer MDA-MB-231 cells (Hsieh et al., 2010a). The nude mice were intraperitoneal injected with lunasin, at 20 mg/kg and 4 mg/kg body weight three times per week for two months, and then transplanted with MDA-MB-231 cells and followed up for the other seven weeks. The tumor incidence was 49% and 33%, respectively, in the two doses of lunasin groups compared to the control group. The tumor generation was significantly reduced in the lower dose of lunasin group by 70% lower relative to control group. Lunasin's inhibitory effect was also found on the tumor weight and volume. In contrast, injection with BBI at 20 mg/kg body weight showed no effect on tumor development. The breast tumor histological sections obtained from the lunasin group showed cell proliferative inhibition and cell apoptosis induction. These mice studies show lunasin as promising alternative to prevent and/or treat skin and breast cancer. Further research should be needed to demonstrate chemopreventive role of this peptide against other types of cancer, as well as to elucidate its *in vivo* mechanism of action.

2.2.3 Lunasin's combinations as a novel strategy against breast cancer

Cancer chemotherapeutic strategies commonly require multiple agents. Combination of two or more chemopreventive agents is becoming the best strategy to prevent and/or treat cancer because of its ability to achieve greater inhibitory effects on cancer cells with lower toxicity potential on normal cells (Li et al., 2005; de Kok et al., 2008). Studies based on molecular mechanisms are needed to optimize this combination, increasing tumor response and reducing toxicity levels in non-cancerous cells (de Kok et al., 2008).

Aspirin (acetylsalicylic acid) has been demonstrated as one of the most promising agents with chemopreventive efficacy against several types of cancer. However, aspirin use has been associated with undesirable side effects, peptic ulcer complications, particularly bleeding and mucosal injury in the stomach, small intestine, and colon (Lanas et al., 2000; Laine, 2006). In an attempt to increase aspirin's efficacy and to avoid its side effects, some researchers have explored the potentially beneficial effects of its combination with several agents that may produce synergisms, resulting in considerably stronger protective effects against carcinogenesis than individually agent use. Since lunasin is present in various seeds and food products, and no safety concerns have been noted. Hsieh and co-workers (2010b) have demonstrated that lunasin promotes the cell proliferation inhibitory and apoptosis inducing activities of aspirin in human breast cancer MDA-MB-231 cells (Hsieh et al.,

2010b). Significant synergistic effects were observed when 10 μM lunasin was combined with 0.5 mM aspirin, resulting in a 73% reduction of cell number. This synergistic effect, at least partially, was mediated through modulating the expression of genes encoding G1 and S-phase regulatory proteins and the extrinsic-apoptosis dependent pathway. Synergistic down-regulatory effects were observed for ERBB2, AKT1, PIK3R1, FOS and JUN signaling genes, whose amplification has been reported as being responsible for breast cancer cell growth and resistance to apoptosis. Moreover, additional studies have demonstrated that lunasin/aspirin combination inhibits foci formation and cell proliferation in chemical carcinogens DMBA and MCA induced-NIH/3T3 cells (Hsieh et al., 2011b). The effect was notably higher than that observed when compounds of the combination acted as a single agent.

Anacardic acid (6-pentadecylsalicylic acid), found in the shell of the cashew nut, has been linked to anti-oxidative, anti-microbial, anti-inflammatory and anti-carcinogenic activities (Kubo et al., 1993; Sung et al., 2008). Synergistic effects have also been observed when lunasin (1–25 μM) was combined with anacardic acid (25 to 100 μM) to treat human breast cancer MDA-MB-231 cells resulting in a concentration-dependent inhibition (Hsieh et al., 2011a). Our findings revealed that lunasin/anacardic acid combination arrests cell cycle in S-phase and induces apoptosis at higher levels than that observed when each compound is used individually. This combination also promotes the inhibition of ERBB2, AKT1/PI3K, JUN and RAF1 signaling gene expression. Importantly, lunasin is demonstrated to promote the anti-carcinogenic properties of anacardic acid, suggesting the role of lunasin is not only in cancer prevention, but also in cancer auxiliary therapy.

Lunasin enhances anti-cancer ability of other chemopreventive compounds from natural or synthetic sources, making them perfect candidate strategies to prevent and/or treat breast cancer. The safety and efficacy of chronic use of these combinations should be further tested in animal models and human studies to establish the optimal dose and duration of treatment. Moreover, studies derived from these findings about mechanisms of action of these lunasin's combinations would open a new vision in the development of novel therapies against breast cancer.

3. Conclusion / Future perspectives

Breast cancer is the most common cancer disease and the leading cause of death in women worldwide. Different food compounds have been demonstrated to be effective against this type of cancer. Among them, peptide lunasin is becoming as one of the most promising agents. This peptide, found in soybean and other plants, has been demonstrated to be bioavailable after resisting gastrointestinal and serum degradation, and to reaches blood and target organs in an intact and active form. Lunasin has been shown to act as a promising agent against breast cancer in both *in vitro* and *in vivo* assays. This peptide inhibits ER-independent breast cancer MDA-MB-231 cells proliferation and promotes other chemopreventive agents' activities, inhibiting proliferation and inducing apoptosis. Moreover, lunasin reduces tumor incidence in a chemical carcinogen-induced mammary tumor and in a xenograft breast cancer mouse model. Moreover, genomics, proteomics and biochemical tools are being applied to complete elucidate its molecular mechanism of action. An array of mechanisms have been revealed for this peptide, including antioxidant and anti-inflammatory properties, histone acetylation inhibitory activity, and modulatory activity of expression of genes and proteins involved in different breast carcinogenesis pathways.

Obtained results from all these studies make lunasin a good candidate for new generation of cancer preventive agents derived from foods. However, there is still much to be learned about lunasin's effects on cancer prevention. The major challenge on the use of lunasin in treating cancer would be the conversion of *in vitro* and *in vivo* results into clinical outcomes. Therefore, it should be needed to design clinical trials that confirm lunasin's chemopreventive properties against breast cancer. Other aspects, such as searching for lunasin in other seeds, optimization of techniques to enrich products with this peptide and studying lunasin's interactions with other food constituents affecting its activity should also be conducted.

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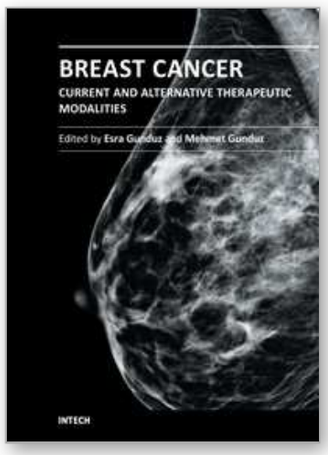

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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In 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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