"Potential Health Benefits of Lunasin: A Multifaceted Soy-Derived Bioactive Peptide"

Vaibhao Kisanrao Lule, Sheenam Garg, Sarang Dilip Pophaly, Hitesh, and Sudhir Kumar Tomar

Abstract: Bioactive peptides are small protein fragments derived from enzymatic hydrolysis of food proteins, fermentation with proteolytic starter cultures, and gastrointestinal digestion. These peptides have positive impacts on a number of physiological functions in living beings. Lunasin, a soy-derived bioactive peptide, is one of the most promising among them. Lunasin encoded within 2S albumin *(GM2S-1)* gene, identified as a novel peptide extracted from soybean seed. It is composed of 43 amino acid residues with a molecular weight of 5.5 kDa. Extensive scientific studies have shown that lunasin possesses inherent antioxidative, anti-inflammatory, anticancerous properties and could also play a vital role in regulating of cholesterol biosynthesis in the body. Its high bioavailability and heat stable nature allow its potential use as dietary supplement. The present review summarizes some of the potential health and therapeutic benefits of lunasin reported hitherto.

Keywords: anticancerous, anti-inflammatory, antioxidative, LDL cholesterol, lunasin

Introduction

Soybean (*Glycine max*) is one of the ancient and major food crops of the Far East. Since the dawn of civilization, oriental people have consumed soybean as a major source of dietary protein and oil. Soybean proves as an excellent source of proteins for vegetarians and is also rich in micronutrients and fiber. Soybean could be processed into variety of food products through various technological and biotechnological interventions. The demographic consumption pattern of soybean varies geographically with Asians utilizing (20 to 80 g/day), mostly in the form of traditional fermented soy foods (Fournier and others 1998; Messina and Flickinger 2002) as compared to Americans consuming meagerly (1 to 3 g/day) (Fournier and others 1998; Cohen and others 2000).

There has been an increasing interest in soybean foods owing to the emerging evidence of its potential remedial role in various disorders like cardiovascular disease, different types of cancer, osteoporosis, and menopausal symptoms. Thanks to their high soy food intake, these effects are particularly palpable among Asian populations (Tikkanen and Adlercreutz 2000; Genovese and Lajolo 2001; Kulling and others 2001; Persky and others 2002). *In vitro* studies, animal trials and epidemiological observations have demonstrated that the consumption of soy phytochemicals and peptides being associated with the reduced episodes of certain cancers (Omoni and Aluko 2005; Gonzalez De Mejia and De Lumen 2006) and an overall reduction in mortality due to prostate (Jacobsen and others 1998; Lee and others 2003), breast (Wu and others 1998; Dai and others 2002; Yamamoto and others 2003), and endometrial cancers (Goodman and others 1997). Bowman-Birk protease inhibitor (BBI) and soy isoflavones are the most widely studied bioactive components. Anticancerous activity of isoflavones is chiefly attributed due to their prolonging estrogenic effects and antioxidant activity (McCue and Shetty 2004) whereas, BBI given the status of investigational new drug by FDA (Food and Drug Administration) in 1992, manifest its effect by inhibiting the protease responsible for the carcinogenesis (Kennedy 1998; Losso 2008). Apart from above bioactive compounds, soy has lunasin-like peptide which may also be credited with prevention and/or treatment of cancer and cardiac disease.

Lunasin (from the Tagalog word "Lunas" for cure) is a unique 43-amino acid peptide sequence encoded within the soybean *Gm2S*-1 gene (Odani and others 1987; Galvez and others 1997). The isolated gene, *Gm2S*-1, codes for a methionine-rich protein and three more peptides: a signal peptide, a small subunit, that is, Lunasin, and a linker peptide (Figure 1). A result of scientific serendipity, discovery of lunasin was a result of collaborative efforts at Dr. De Lumen's lab at UC Berkeley in year 1996. They, for the first time, reported that although the peptide is found in small quantity, it can block cell division by binding to specific chromosomal proteins called "hypoacetylated histones." Further investigations on the peptide revealed an important physiological activity of lowering cholesterol (Lunasin: *http://www.lunasin.com/About.aspx*; Galvez 2012). Besides its potential anticancerous and cholesterol lowering properties, lunasin is also an effectual antioxidant and anti-inflammatory agent. The present review focuses on the potential health benefits of lunasin reported to date.

Potential Health Benefits of Lunasin

Anticancerous activity of lunasin

Chemopreventive property of lunasin was endorsed by both *in vitro* and *in vivo* methods. In absence of carcinogens, exogenously supplemented lunasin does not have any visible impact on cellular morphology but, selectively hampers transformation in their presence (Lam and others 2003). Preliminary studies on its biological activity revealed that lunasin resulted in mitotic arrests leading to cell death by visible binding to kinetochore regions of the centromere and obstructing microtubule attachment and form nonseptated filaments in *E. coli* (Galvez and Benito 1999). These initial investigations suggested the possible anticancerous activity of lunasin and could be useful as a therapeutic agent. Application of synthetic lunasin to mammalian cells obstructed cellular transformation by chemical carcinogens and viral oncogenes Ras and E1A, but the peptide exposure neither affected

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normal immortalized cells nor cancer built cell lines (Galvez and others 2001; Jeong and others 2002; Lam and others 2003; Hernandez-Ledesma and others 2009a). This assumption was further strengthened by *in vivo* study showing suppression of skin papilloma development SENCAR (SENsitivity to CARcinogenesis) mice treated with combination of chemical carcinogen, namely, 7,12-dimethylbenz(a) anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) (tumor promoter) on topical application of lunasin (Galvez and others 2001). In the presence or absence of DMBA, lunasin acts as an antimitotic agent in SENCAR mice by delaying induction of tumors in epidermal cells producing keratin, detected by ^2H_2O labeling technique (Hsieh and others 2004). Similar observations existed in mouse xenograft model and various cancer cell lines (Dia and Mejia 2010; Gonzalez De Mejia and others 2010; Hsieh and others 2010a, 2010b). Anticancerous mechanism of lunasin (Figure 2) involves disruption of the dynamics of normal histone acetylation and deacetylation by specific binding to core deacetylated histones (H_3 and H_4), thereby selectively kill the tumor cells generated, due to inactivation of Rb, p53, and pp32 (tumor suppressor proteins) (Galvez and others 2001; Jeong and others 2002; Lumen 2005; Jeong and others 2007c; Hernandez-Ledesma and others 2011). Further studies demonstrated that anticancerous property of lunasin also associated with alleviation of certain proinflammatory markers by suppressing NF-kB pathway (Dia and others 2008; Gonzalez De Mejia and Dia 2009; Dia and Mejia 2010; Gonzalez De Mejia and others 2010; Dia and De Mejia 2011a, 2011b). Gene expression studies revealed that application of lunasin in different cell culture model influence various signaling pathways and its biological activity to be independent of histone acetylation (Dia and De Mejia 2011c). Immunostimulatory cytokines are not only known for their antitumoral activity but also enhances antitumor immunity and serve as a part of cancer therapeutics in the form of cytokines immunotherapy. Lunasin synergistically works with cytokines (IL-12 or IL-2) and improves the tumoricidal activity of natural killer (NK) cells in *in vitro* and *in vivo* tumor models (Chang and others 2014). Recently, significant antineoplastic effects of lunasin and its role in modulating the various pathways involved in different systemic cancers was demonstrated (Kapoor 2014). Though the potential anticancerous effect of lunasin was studied extensively in the last decade, there has been a little progress to verify its *in vivo* efficacy in animals or humans. Jones and Srivastava (2014) reviewed suitability of Drosophila as a model organism using sophisticated genetic tools to study anticancer effects of lunasin and proposed a new way in lunasin research to prove its *in vivo* efficacy.

Hypothesized role of lunasin in cholesterol reduction

In 1999, United States Food and Drug Administration (USFDA) validated a claim that soy products were "heart healthy." Afterwards, the American Heart Association (AHA) in February 2006 released a scientific advisory report on soy protein, isoflavones, and cardiovascular health followed by analyzing clinical data published (Sacks and others 2006). The clinical outcome of the experiments involving soy proteins have prompted the FDA to allow a health claim on food labels stating that consuming 25 g soy protein/day may lower the cardiovascular problem. There is considerable epidemiological evidence that dietary factors from soy such as soy proteins and its nonprotein components, saponins and isoflavones (for example, genistein and diadzein) can help to manage cholesterol levels and lower coronary heart disease (CHD) risk in certain individuals and animals (Sirtori and others 1993; Sagara and others 2004; Wang and others 2004; Zhong and others 2007). Epidemiological studies have linked soy food consumption with decreased risk of cardiovascular disease in some Asian populations (Adlercreutz and Mazur 1997). A large-scale cohort study among Chinese women with nonfatal myocardial infarction showed an inverse relation between soy food intake and risk of CHD in a dose-response manner (Zhang and others 2003). Meta-analysis results from 38 clinical studies, including 730 research volunteers, showed that soy protein intake was associated with significant decrease in the levels of bad cholesterol and a nonsignificant increase in good cholesterol level (Anderson and others 1995). *In vivo* study in rats revealed that supplementation of 7S globulin, a major storage protein in soybean, significantly reduced cholesterol concentration in plasma by 35% (Sirtori and others 1993). In contradiction, there are some studies which also revealed that components like nonprotein fraction, saponins and isoflavones do not show any positive effect in lowering cholesterol (Greaves and others 1999; Adams and others 2004). The evidence supports that soy protein is the responsible nutrient and not soy isoflavones. Although FDA has approved consumption of soy protein as heart healthy, however, soy protein fraction contributing toward this effect is yet to be screened out. Transcriptional activation of HMG Co-A reductase (rate limiting enzyme) via specific acetylation of histone H3 by P300/CBP-associated factor (PCAF) is an essential step in hepatic cholesterol biosynthesis. In relation to this, lunasin works in two ways to reduce the serum

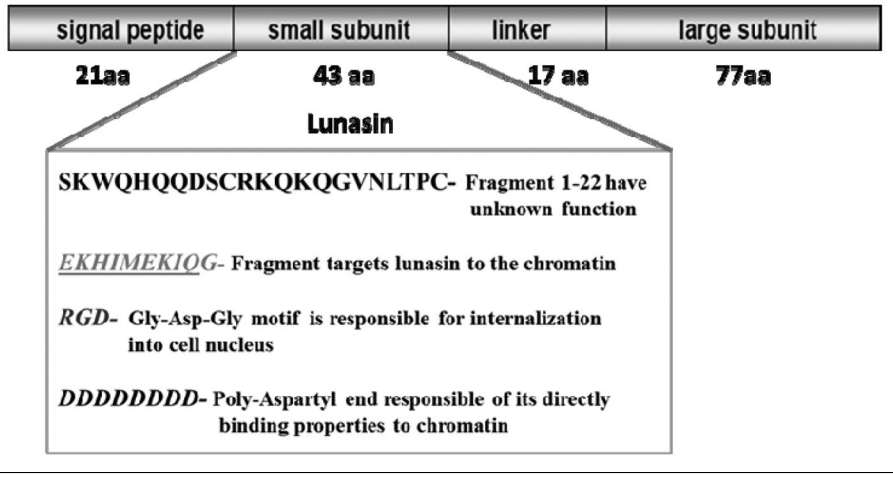

Figure 1–Lunasin: small subunit of a 2S albumin (*Gm2s-1 encoded*) protein in soybean seeds.

LDL cholesterol levels. First, it selectively disrupts a requisite step in the production of HMG-CoA reductase (Figure 3). Lunasin reduces acetylation of the histone H3 tail at K14 position by PCAF, thus lowering the HMG-CoA reductase gene expression and make it unavailable for the liver to conduct cholesterol synthesis (*Lunasin*: *http://www.lunasin.com/About/CardiovascularResearch*). Second, lunasin increases expression of the LDL-receptor gene (Figure 4), which increases quantity of receptors to clear LDL cholesterol from bloodstream. In the presence of lunasin, the levels of SP1 proteins (the coactivator of SREBP, that is, Sterol Regulatory Element-Binding Protein for LDL-receptor production) increased two times higher as compared to without lunasin (*Lunasin*: *http://www.lunasin.com/About/CardiovascularResearch*; Galvez 2010, 2012). *In vitro* studies in HepG2 liver cells revealed that lunasin significantly downregulated HMG Co-A reductase expression by inhibiting PCAF acetylation of H3-Lysine 14 and enhances the ordered expression of SP1 proteins (Galvez 2010, 2012; Galvez and others 2014). Further, an *in vivo* study revealed that supplementation of lunasin-enriched soy extract (LSE) with casein lowered LDL cholesterol level as compared to only casein fed diet in pigs having mutated LDL receptor gene (Galvez 2012). Recently, Galvez (2013) granted a patent, providing method for reducing cholesterol level in an individual consuming product containing an effective amount of lunasin and claims it to reduce total cholesterol, LDL cholesterol, and overall lipid profile in an individual.

Statin drugs (acclaimed cholesterol lowering drugs) manifest their effect by blocking the HMG-CoA reductase enzyme activity, therefore claiming a cholesterol lowering effect (Galvez 2012).

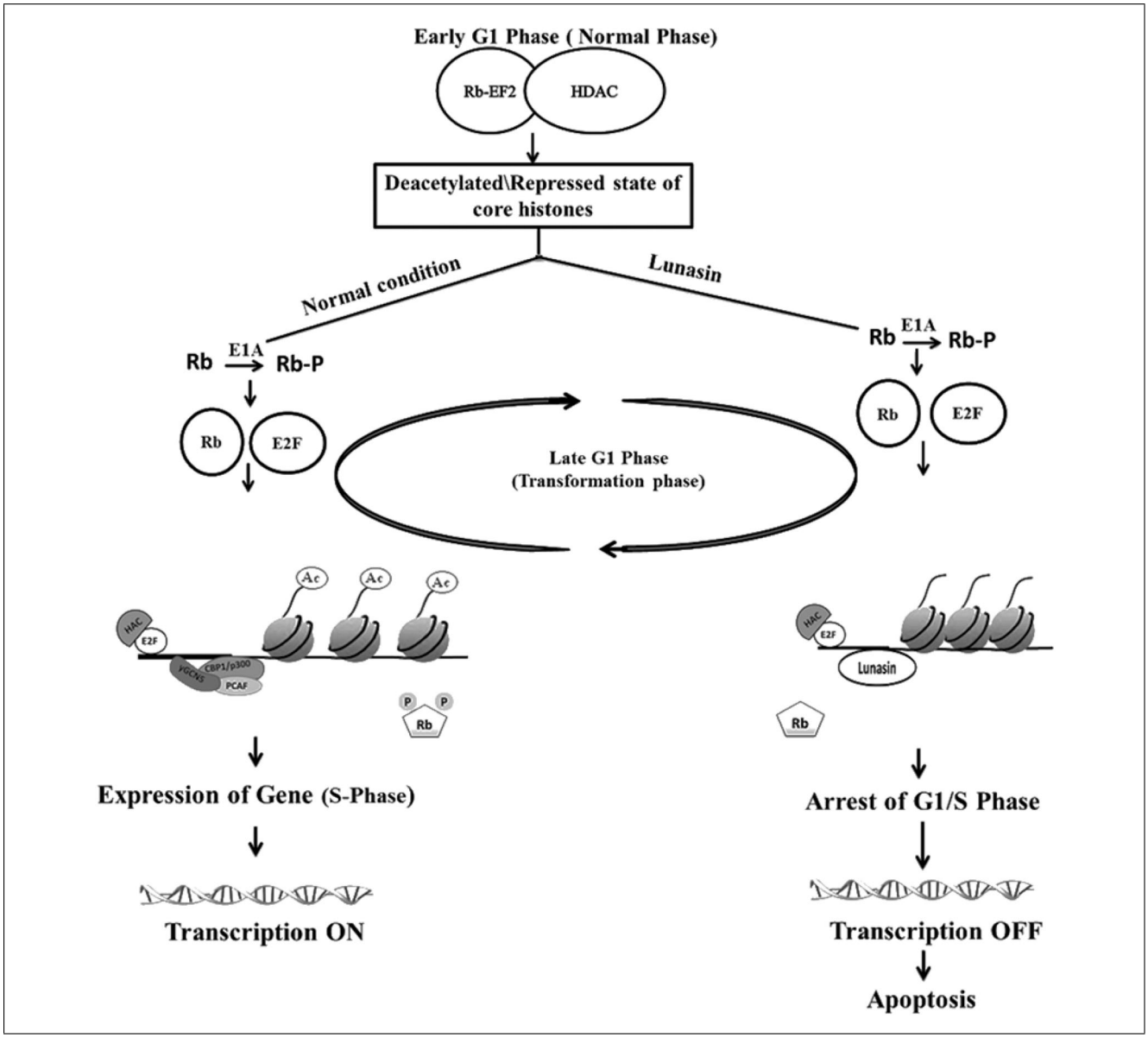

Figure 2–Mechanism of action of lunasin as cancer-preventive agent: Rb and Rb-p are the retinoblastoma protein, represents unphosphorylated and the phosphorylated forms, respectively. During early G1 phase in normal cells, Rb-E2F complex recruits histone deacetylases (HDAC) keeping the core histones in the deacetylated (repressed) state. Cell being transformed in the late G1 phase, E1A inactivated Rb by phosphorylation, dissociates the Rb-E2F complex, exposing the deacetylated histones to the histone acetyltransferases enzymes which carries core histone acetylation (PCAF, CBP/p300 and yGCN5). Histone acetylation allows the expression of genes that encodes products needed for S-phase, activating the cell cycle progression. Lunasin competes with the histone acetyltransferases in binding to the deacetylated histones, switching off the transcription, perceived by cells as abnormal state, leads to arrest G1/S phase and caused apoptosis.

The effect of lunasin is identical to statins but differs in the mode of action, as it seems to inhibit expression of HMG-CoA, which leads to increased LDL receptor expression at transcriptional level and not inhibiting its enzyme activity (Galvez 2012; Galvez and others 2014). Moreover, as compared to lunasin, statins can often be too efficient and block too much of the HMG-CoA enzyme, which culminates into serious side effects like termination of cellular functions (*Lunasin*: *http://www.lunasin.com/About/CardiovascularResearch*). It is, however, important to note that cholesterol-lowering property of lunasin has been studied in cell lines and animal models and the attempts need to traverse clinical studies to conclusively prove its efficacy and safety in therapeutic application.

Antioxidant and anti-inflammatory properties of lunasin

Chronic inflammation and oxidative stress are considered the most critical factors in the cancer development, resulting in approximately 15% to 20% of malignancies worldwide (Kuper and others 2000; Allavena and others 2008). It was postulated that initiation in cascade of inflammatory events contributes to abnormal growth of tissue which ultimately results in tumor invasion and metastasis (Khan and others 2008). Determination of anti-inflammatory and/or antioxidative properties might serve as a good index for screening any antitumorigenic agents (Tsai and others 2005; Villegas and others 2008). Lunasin displays antioxidative/anti-inflammatory property in versatile manner: (a) inhibit 2, 20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt radical scavenger, (b) inhibit ROS production, (c) inhibit the release of proinflammatory cytokines (tumor necrosis factor-a [TNF-a] and interleukine-6 [IL-6]) (Hernandez-Ledesma and others 2009b). Recently, *in vitro* antioxidative activity of lunasin peptide demonstrated it to be a potent scavenger of peroxyl and superoxide radicals. In addition, the protective role of lunasin on cell viability and antioxidant defenses of human Caco-2 cells challenged by hydrogen peroxide and tert-butylhydroperoxide was also evaluated. This peptide remained partially intact during incubation time and prevented the cells from oxidative damage induced by both chemical agents (Garcia-Nebot and others 2014). Most recently, Fernandez-Tome and others (2014) studied the stability of lunasin in human liver HepG2 cells, and its chemoprotective effect against oxidative stress induced by *tert*-butylhydroperoxide. Pretreatment of cells with the lunasin (0.5 to 10 μM) significantly prevents the ROS generation, glutathione peroxidase, and catalase activities. Lunasin might confer a significant chemoprotection against oxidative stress-associated liver disorders by restraining ROS overproduction. *In vitro* study depicts the antioxidative property of lunasin in oats (Avena sativa L) and demonstrated it to be similar to the synthetic peptide (Nakurte and others 2013). It was found that *RGD* motif in lunasin and similar peptides regulated the inflammatory-related pathologies by inhibiting Protein Kinase B (Akt)-mediated NF-κB pathways through interaction with $\alpha V\beta 3$ integrin in

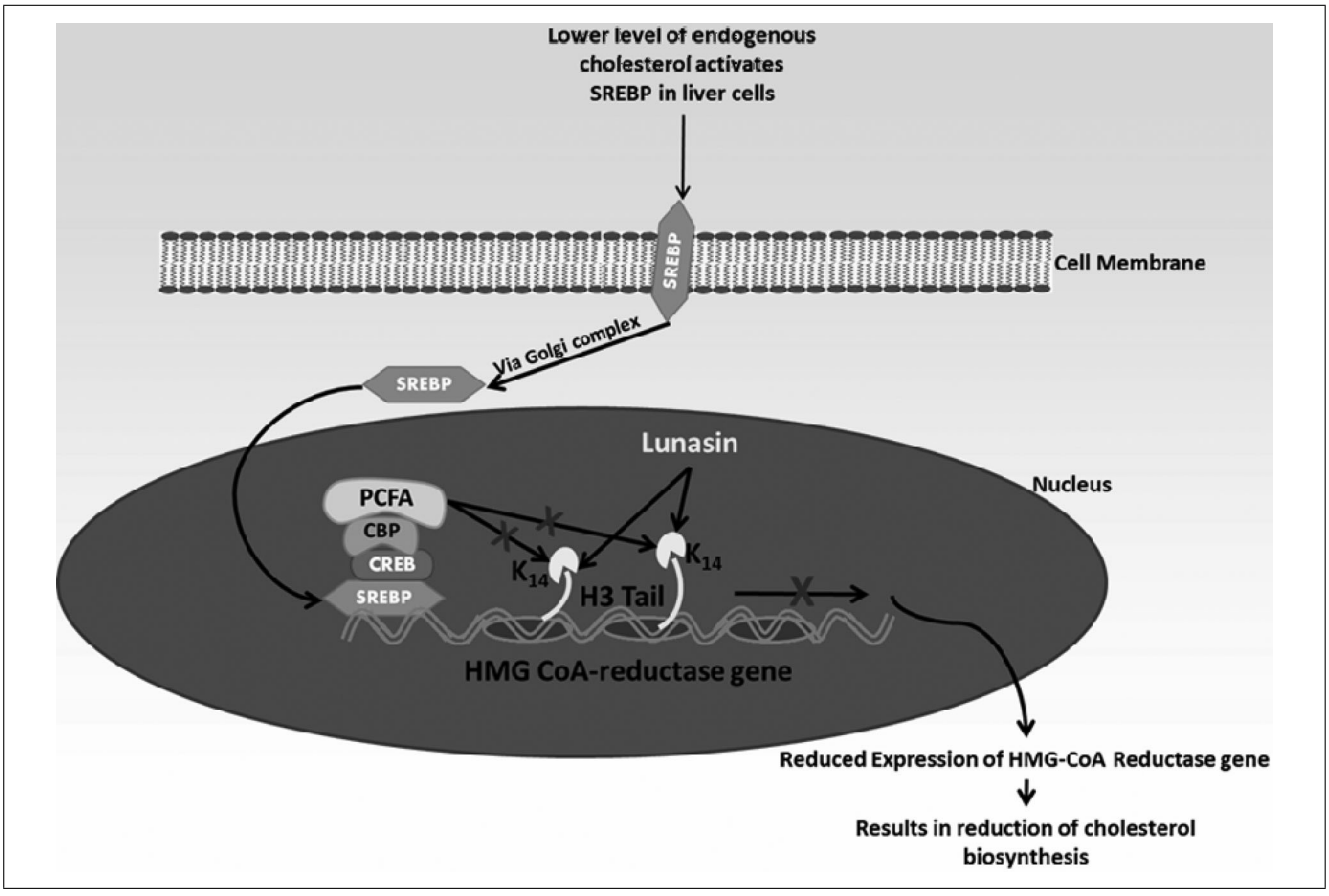

Figure 3–Role of lunasin in reduction of cholesterol biosynthesis: lunasin reduces total and LDL cholesterol level by binding to deacetylated histone H3 (specifically lysine 14 in H3 N-terminal tail) and inhibiting histone H3 acetylation by PCAF (through its association with the CREB-binding protein), thereby reducing SREBP (Sterol Regulatory Element-Binding Proteins which are transcription factors that bind to the sterol regulatory element DNA sequence) activation of the HMG CoA reductase gene resulting in lower endogenous cholesterol biosynthesis (adapted and modified illustration from http://www.lunasin.com).

lipopolysaccharide (LPS)-induced human THP-1 macrophages, thus involving antagonism of integrin signaling and downstream proinflammatory cascades (Cam and De Mejia 2012). Thereby, lunasin could be a good therapeutic agent in combating various inflammatory related disorders. Recently, it also has been proved that lunasin acts as an adjuvant by targeting dendritic cells (DCs) and modulate the immune responses to vaccine antigens in human peripheral blood mononuclear cells (Tung and others 2014).

Bioavailability of lunasin

Adaptability to different delivery systems is an essential feature for therapeutic molecules with orally administrable molecules better suited for drug and food formats. They should resist degradation by the gastric enzymes and be able to reach the target tissue in an intact form (Park and others 2005; Park and others 2007). Lunasin was found to resist the harsh environmental condition of gastrointestinal tract which was attributed to the protection provided by BBI (Hernandez-Ledesma and others 2009b). Moreover, *in vitro* and *in vivo* bioavailability studies suggested that the combined protection of lunasin contributed by BBI and other natural protease inhibitors in soy and wheat protein plays a crucial role in preventing degradation of the peptide to make it bioavailable (Jeong and others 2007c). Therefore, high percentage of daily ingested peptide reaches to target organs and tissues in a biologically active form (Hsieh and others 2010b). Further human trial revealed that consumption of soy protein in healthy men resulted in an absorption rate of about 4.5% from the total lunasin ingested from 50 g of soy protein (Dia and others 2009; De Mejia and Dia 2010).

Lunasin Content in Soybean, Cereals, Medicinal Plants, and Commercial Soy Products

Soybean is rich source of lunasin, with concentration ranging from 4.4 to 70.5 mg lunasin/g of soy protein in different genotypes of soybean (Hernandez-Ledesma and others 2009a), which increases notably during seed maturation and tends to decrease during sprouting with respect to soaking time (Park and others 2005). Interaction of cultivars, growing temperature, and soil moisture conditions significantly affect the lunasin concentration (Wang and others 2008). Content of lunasin among soybean cultivars varied significantly, evincing new possibility to breed novel high lunasin containing soybean varieties (Gonzalez De Mejia and others 2004; Wang and others 2008). Lunasin concentration also varies (12 to 44 mg lunasin/g of flour) in different commercially available soy protein produced by large-scale processing methods (Gonzalez De Mejia and others 2004). The least concentration of lunasin was found in defatted soy flour (5.5 mg/g protein) followed by soy isolate (6.9 mg/g protein) and then soy concentrate (16.5 mg/g protein) (Jeong and others 2003). These data could be useful in

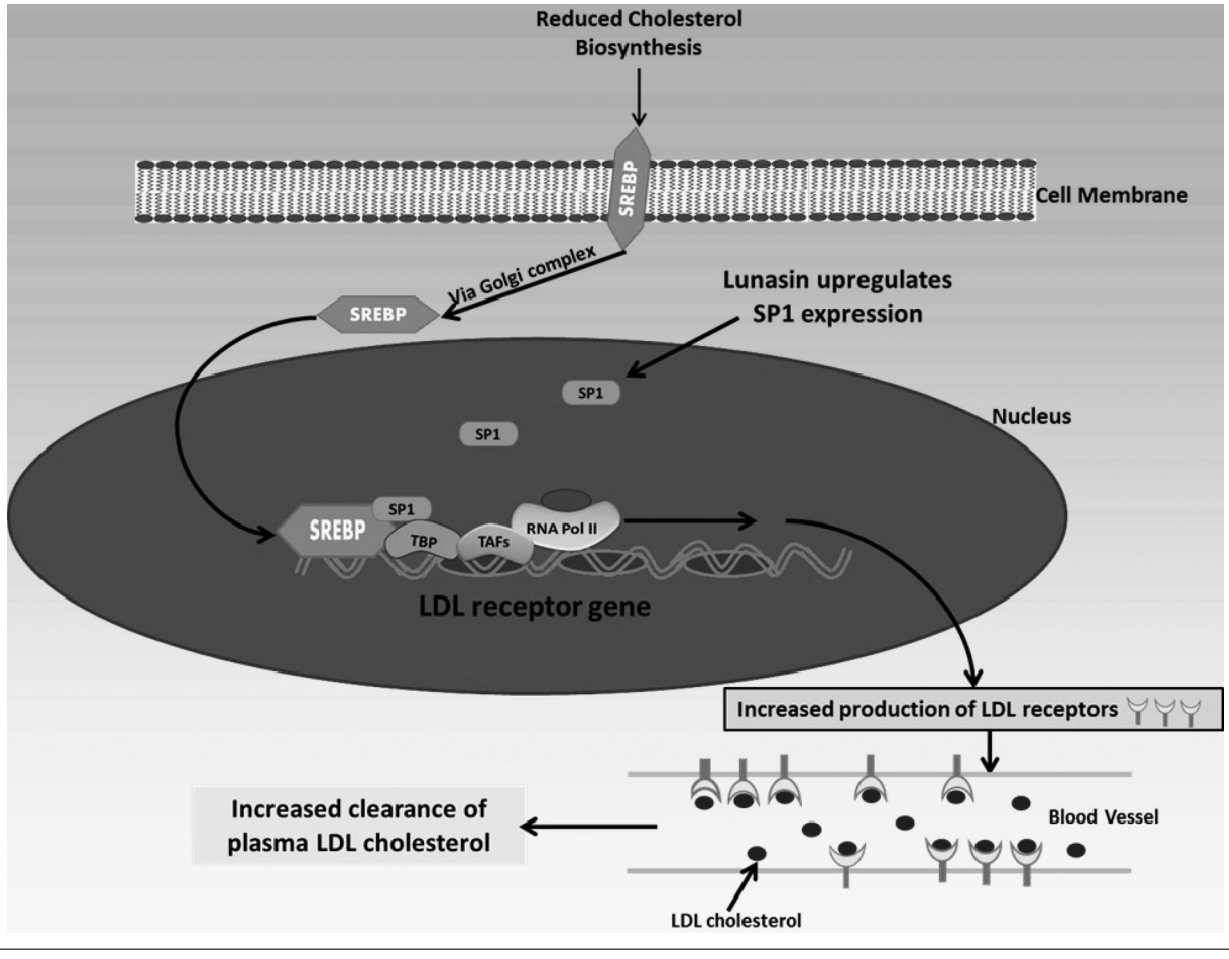

Figure 4–Role of lunasin in upregulation of LDL-receptor gene: lunasin increases the expression of SP1 (co-activator of SREBP, that is, sterol regulatory element-binding proteins for LDL-receptor production). In presence of increased SP1 that radially bind to SREBP, there is more efficient production of LDL receptors, this enhances clearance of plasma LDL cholesterol (adapted and modified illustration from http://www.lunasin.com).

Table 1–Lunasin concentration in soybean seeds, cereals, medicinal plants, and commercial soy preparations.

Seeds/plants/products (common name/s)	Lunasin content		Techniques		References
	mg lunasin/g protein	mg lunasin/100g seedor product	Separation	Identification	
Soybean	4.4 to 70.5	50 to 810	Anion-exchange chromatography immunoaffinity column chromatography	Western blot analysis and mass spectrometric peptide mapping	Gonzalez De Mejia and others 2004
Barley	5.9 to 8.7	1 to 2	Ion-exchange chromatography immunoaffinity column chromatography	SDS-PAGE Western blot MALDI (matrix-assisted laser desorption ionization) peptide mass mapping	Jeong and others 2002
Wheat	–	20 to 30	Liquid chromatography	Western blot analysis and electrospray ionization mass spectrometry (LC-ESI-MS)	Jeong and others 2007a
Rye	–	4.5 to 15	Ion-exchange column chromatography and reverse phase HPLC	HPLC-based identification and Western blot analysis for quantification (software UN-SCAN-IT gel version 5.1)	Jeong and others 2009
Oat	–	19.5 to 19.7	Reverse phase HPLC	Electrospray ionization tandem mass spectrometry (LC-ESI-MS)	Nakurte and others 2013
Amaranth	9.5 to 12.1	–	Immunoprecipitation prior to identification assays	ELISA, Western blot analysis, and MALDI-TOF peptide mass mapping	Silva-Sanchez and others 2008
Bladder-cherry, winter cherry	17.0	10	Membrane dialysis, ion-exchange column chromatography, and reverse-phase HPLC	HPLC-based identification and Western blot analysis for quantification (software UN-SCAN-IT gel version 5.1)	Jeong and others 2007b
Sunberry, wonderberry, Black nightsade	36.4	180	Membrane dialysis, ion-exchange column chromatography and reverse-phase HPLC	HPLC-based identification and Western blot analysis for quantification (software UN-SCAN-IT gel version 5.1)	Jeong and others 2007b
Jimson weed, devil's trumpet, devil's seed	10.3	30	Membrane dialysis, ion-exchange column chromatography, and reverse-phase HPLC	HPLC-based identification and Western blot analysis for quantification (software UN-SCAN-IT gel version 5.1)	Jeong and others 2007b
Hyyodori-jogo (Japanese name)	22.3	40	Membrane dialysis, ion-exchange column chromatography, and reverse-phase HPLC	HPLC-based identification and Western blot analysis for quantification (software UN-SCAN-IT gel version 5.1)	Jeong and others 2007b
Different commercial regular soymilk samples	1.1 to 2.7	2.3 to 9.2	Ion-exchange chromatography, membrane dialysis, and ultrafiltration	SDS-PAGE, Western blot analysis, ELISA, and LC/MS-MS	Cavazos and others 2012
Different commercial organic soymilk samples	0.9 to 2.7	2.9 to 9.0	Ion-exchange chromatography, membrane dialysis, and ultrafiltration	SDS-PAGE, Western blot analysis, ELISA, and LC/MS-MS	Cavazos and others 2012
Different soy formula samples	4.3 to 5.4	7.3 to 8.9	Ion-exchange chromatography, membrane dialysis, and ultrafiltration	SDS-PAGE, Western blot analysis, ELISA, and LC/MS-MS	Cavazos and others 2012
Different high protein soy shake samples	0.08 to 0.26	1.2 to 3.6	Ion-exchange chromatography, membrane dialysis, and ultrafiltration	SDS-PAGE, Western blot analysis, ELISA, and LC/MS-MS	Cavazos and others 2012

Table 2–Commercially available preparations of lunasin and their health claims.

Brand name	Company	Nature	Country	Application	Health claims
LunaSoy™	Soy Labs®, LLC	Powder (protein complex)	USA (Jefferson City, Missouri)	Dietary supplement and as an ingredient for development of functional foods and nutraceuticals	• Heart health • Immune modulation • Skin health • Anti-inflammatory • Antiaging
Lunasin XP®	Soy Labs®, LLC	Powder (peptide extract)	USA (Jefferson City, Missouri)	Dietary supplement and as an ingredient for development of functional foods, beverages, and nutraceuticals	• Antiaging • Heart health • Immune modulation • Skin health • Anti-inflammatory
LunaRich®	Reliv International	Capsules (Highly pure and concentrated form)	USA	Dietary supplement	• Support weight loss • Heart health • Overall cellular health • Improve immunity • Metabolic wellness
FSP100™	Carefast® Products, Inc.	Soy drink mix	USA (Las Vegas)	Food supplement	• Heart healthy • Highly nutritious
Soy Guard™	Biotec-Foods	Capsules	USA	Dietary supplement	• Highly bioavailable form of phytonutrient complex providing a complete spectrum of soy phytonutrients including genistein, daidzein, and phyto-estrogens (lunasin)

recommending dietary intake of lunasin as well as for developing the soy-based foods enriched with lunasin. Since its first discovery in soybean, lunasin has also been reported to be present in wheat, barley, oats (cereal grains known for their physiological effects), *Solanum nigrum* (Jeong and others 2007b; Jeong and others 2010), and Amaranth (Silva-Sanchez and others 2008). Amaranth is a highly nutritious traditional Mexican plant. Besides providing grains and leaves, it is also known for exerting certain physiological effects (Silva-Sanchez and others 2008). However, recent searches of transcript and DNA sequence databases for wheat and other cereals did not reveal the sequence encoding the soybean lunasin or similar sequences and end up with fact that lunasin was not a cereal protein but may have arisen as a result of contamination with another organism (for example, fungal) (Mitchell and others 2013). In order to establish the presence and absence of lunasin in wheat varieties, most recently Dinelli and others (2014) conducted a study based on chemical (LC-ESI-MS) and molecular (PCR) analysis and revealed that no lunasin-related sequences were found by both methods in the analyzed 12 selected wheat varieties. Jeong and others analyzed the seeds of several oriental herbal medicinal plants and reported that lunasin was found in all plants under *Solanaceae* family, except *L. chinensis* (Jeong and others 2007c). Moreover, good amount of lunasin also found in commercially available soy preparations (Table 1). Lunasin concentration may vary according to product storage condition, method of preparation, environmental factors, and type of soy genotype used in manufacturing the product. Lunasin concentration in different soy preparations has been extensively detailed by Cavazos and others (2012). Recently, considerable amount of highly stable lunasin was reported in oats (Avena sativa L) (Nakurte and others 2013). A more comprehensive search of lunasin and its homologue is still going on in different seeds and other herbal plants in order to develop a relationship between presence and taxonomic properties of the plants for this multifaceted peptide. Lunasin concentration in soybean, cereals, medicinal plants, and commercial soy preparations is indicated in Table 1.

Lunasin Production

Production of lunasin in highly purified form is cost intensive, limiting its commercial applications as well as scientific studies. Lack of proper methods is a major hindrance to get lunasin in its concentrated or purified form (Seber and others 2012; Hernandez-Ledesma and others 2013). Recently, Cavazos and others (2012) have developed a method resulting in >90% purity of lunasin from defatted soy flour. However, Seber and others (2012) developed an improved resulting >99% purity of lunasin from same source by the application of more advanced techniques, namely, anion-exchange chromatography, ultrafiltration, and reversed-phase chromatography. Separation and identification methods employed for lunasin extraction from various sources are enlisted in Table 1. Most preferably lunasin is produced by two methods, either by purification from natural sources or conventional chemical synthesis; an alternative to these two methods is recombinant production of lunasin by transgenic organisms which could be a novel approach for its efficient and cost-effective production (Setrerrahmane and others 2014). In view of this, recombinant production of lunasin with a yield of 75 mg/L and 210 mg/L in *E. coli* and *Clostridium thermocellum* was explored (Kyle and others 2012; Setrerrahmane and others 2014). Lunasin was successfully expressed as recombinant his-tagged gene in *E. coli* and was found to have identical biological activity with synthetic lunasin (Liu and Pan 2010). Recently patent has been granted for the method of preparation of lunasin in plants which includes production of lunasin in plant by expressing a fusion protein comprising lunasin and cleaving it from the plant fusion protein (Davis and others 2014). Production of lunasin by recombinant technology by harnessing these potential cell factories could serve as an effective alternative over conventional chemical methods.

Role of Microbial Fermentation in Lunasin Synthesis

Fermentative and proteolytic activity of sourdough lactic acid bacteria (LAB) determines not only the sensory, technological, and nutritional characteristics but also the functional features of the resulting baked foods (Gobbetti and others 2005). Rizzello and others (2012) recently studied the potential role of microbial proteolytic system for increasing the lunasin concentration in food matrices by sourdough fermentation. The study investigated increase in lunasin content in different flours when fermented by LAB as compared to control doughs and the highest concentration of lunasin (2 to 4 times) was reported in sourdough fermented by *Lactobacillus curvatus* SAL33 and *Lactobacillus brevis* AM7. In this perspective, a study suggested new possibilities of its cost-effective bio-production over chemical synthesis and the development of innovative functional fermented foods (Rizzello and others 2012).

Commercially Available Preparations of Lunasin

In recent years, lunasin have been used to develop series of functional foods by addition of peptide extract, protein complexes, and dietary supplements. LunaSoy™ (protein complex) and Lunasin XP® (peptide extract) are the two soybean-derived commercial preparation being used as a potential ingredients for formulating the cholesterol lowering functional foods (Udenigwe and Aluko 2011; Soy Labs 2014). LunaRich® is the line of products containing optimized bioactive lunasin from Reliv International Company available in the market as dietary supplements for heart and overall cellular health. A list of similar commercially available lunasin products or preparations with their claimed health benefits is given in Table 2.

Conclusions

Lunasin, a novel soy peptide, serves as promising candidate in exerting multifaceted health-promoting effects like anti-inflammatory, cholesterol lowering, anticancerous, and antioxidative activity. Lunasin content varies according to different seed varieties, development stages, and processing parameters. Resistance of lunasin to the gastric enzymes preserves its functionality and hence contributes toward its bioavailability. All the aforesaid factors make lunasin one of the most sought molecules for future therapeutic research but it is imperative to assemble information on mechanistic inside of its role in the host system. There is a need to use advanced molecular and proteomic tools to decipher its molecular mechanisms of action in various diseases and for establishment of its safety and efficacy.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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