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Lunasin: A promising polypeptide for the prevention and treatment of cancer (Review)

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Abstract. Strategies for the treatment of cancer remain unsatisfactory due to the poor understanding of the complicated underlying molecular mechanisms of carcinogenesis. A number of types of cancer exhibit a marked association with dietary habits and lifestyles. Therefore, the modulation of dietary habits or lifestyles may be an effective strategy for preventing the formation and progression of cancer. Proteins and polypeptides from soybean have been developed as healthcare products due to their marked activity in inhibiting the progression of cancer at various stages. Lunasin, containing 43 amino acid residues, is one such example of a soybean-derived polypeptide that has been demonstrated to exhibit marked anti-cancer activity. In the present review, studies of the underlying molecular mechanisms and potential advantages of lunasin in the prevention and treatment of cancer have been examined, to provide a theoretical reference for the development of natural product-based agents or healthcare products for the prevention and treatment of cancer.

Contents

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1. Introduction

Cancer is well-known to be one of the leading causes of mortality worldwide (1). However, the therapy of various types of cancer remains unsatisfactory due to the poor understanding of the complicated underlying molecular mechanisms of carcinogenesis. A number of epidemiological studies have demonstrated that a number of types of cancer exhibit a marked association with dietary habits and lifestyles (2-4). A statistical study has demonstrated that ~3.07 million individuals in China were diagnosed with cancer in 2012, which accounts for 1/5 of the total number of patients with cancer worldwide (5). Furthermore, ~2.21 million patients succumbed to cancer in China, which is ~25% of the total mortality worldwide due to cancer (5). Epidemiological evidence has demonstrated that cancer in 35% of patients exhibits a marked association with lifestyle, particularly diet (2). Therefore, the adjustment of dietary habits and lifestyles may be an effective strategy to prevent carcinogenesis. Cancer cell- and tumor-bearing animal models have demonstrated that the consumption of foods containing natural compounds with anti-cancer activity is able to markedly reduce the risk of cancer, and increase the sensitivity of cancer cells to treatment (6). A number of phytochemicals, including resveratrol, quercetin and flavonoids, have been demonstrated to exhibit marked anti-cancer activity (7-9). Similarly, foodborne proteins and polypeptides have attracted attention due to their specific advantages as anti-cancer substances (10,11). Compared with certain small-molecule drugs, polypeptides exhibit characteristics of increased affinity, marked ability of specific targeting and decreased toxicity; furthermore, polypeptides exhibit increased permeability in tissues compared with protein-based drugs (12). Therefore, polypeptides have been recognized as potential natural anti-cancer substances for inhibiting the development and progression of cancer at different stages (13).

Following extensive exploitation and utilization of natural products, soybean and other associated products have aroused interest from consumers due to their health-promoting benefits (14). Previous studies have demonstrated that increased soybean consumption in Asian populations is associated with decreased incidence

of osteoporosis, cardiovascular disease and cancer (15-17). Furthermore, soybean products may reduce the risk of developing prostate and breast cancer (18,19). Soybean was demonstrated to contain a variety of bioactive compounds, including protease inhibitors, lunasin, sitosterol, saponins and isoflavones, with significant anti-cancer activity (20,21). Bowman-Birk protease inhibitor (BBI) and isoflavones have been investigated extensively (18,20,22). Similarly, lunasin, isolated and extracted from soybean or other similar plants, has been reported as a polypeptide with a clear function to inhibit chemical-induced carcinogenesis (14). In the present review, studies of the underlying molecular mechanisms and corresponding advantages of lunasin for the prevention and treatment of cancer have been systematically examined to provide a theoretical reference, for the development of natural product-based agents or healthcare products, for the prevention and treatment of cancer.

2. Structure of lunasin

Lunasin, a polypeptide with molecular mass of 5.5 kDa, is able to enter mammalian cells within min and target nuclei within 18 h (23). Lunasin contains 43 amino acid residues, which comprise a fragment with an unknown function (residues 1-22), a helical structure (residues 23-31) that is similar to the conserved structure of chromatin-binding protein for binding to histones H3/H4, a cell adhesion module of Arg-Gly-Asp (RGD) (residues 32-34) that functions as an extracellular matrix to promote the intracellular accessibility of lunasin and a critical fragment of nine aspartic acids at the C-terminus that is involved in anti-mitotic functions (23). The full sequence of lunasin is SKWQHQQDSCRKQLQGVNLT PCEKHIMEKIQGRDDDDDDDDDD and the fragments are depicted in Fig. 1 (14).

3. Characterization of lunasin

Lunasin has been identified in soybean, barley, wheat and rye, and has been subjected to corresponding comparative analysis (24,25). However, due to the genotypic impact of different species, the lunasin content varies between species (26). The lunasin content in soybean, barley, wheat and rye was determined to be between 0.5 and 8.1, between 0.013 and 0.099, between 0.2 and 0.3, and between 0.045 and 0.150 mg/g seed, respectively (14,25,26). Therefore, soybean and wheat are recognized as the principal sources of lunasin (14).

The common extraction procedures for lunasin include crushing, defatting, extraction, dialysis, centrifugation and determination (14). This extraction method usually results in a crude protein containing lunasin, which requires further purification. Ion-exchange column chromatography has been used for the purification of lunasin extracted from soybean and barley in previous studies, and the principal material used in the ion-exchange column is bio-gel resin AG1-X4 with a particle size of between 100 and 200 mesh, and the volume of the ion-exchange column is 5.0x50 cm with a filled height of 40 cm (27,28). In addition, gel electrophoresis, western blotting and mass spectrometry are also used for the qualitative analysis and quantitative determination of lunasin (29,30).

4. Molecular mechanisms and advantages of lunasin, in the prevention and treatment of cancer

Advantages of lunasin in the prevention and treatment of cancer. An important characteristic of the ideal agents or health-promoting products for the prevention and treatment of cancer is their marked bioactivity in the inhibition of cancer cell generation and growth. Following oral administration of lunasin, it is absorbed into the bloodstream and reaches target tissues or organs in an active and stable state (31). Animal studies using rats fed on a soybean diet rich in lunasin demonstrated that the marked stability of lunasin enables it to reach target tissues and organs following administration, thus serving an important role in the prevention of cancer (24,32). The bioactivity of lunasin has been investigated in humans, and results have demonstrated that 4.5% lunasin at full activity is able to enter the plasma of healthy volunteers in 30 min (33). In spite of the marked bioactivity of lunasin following administration, another study has demonstrated that BBI is required to protect lunasin from digestion (34). This protective role provides the favorable conditions required for lunasin to perform its functions. In addition, its marked stability following administration may be due to its specific secondary or tertiary structure, which remains unclear and requires further study. These studies provide the scientific basis for understanding the functions of lunasin in the prevention and treatment of cancer, and provide a rationale for the development of lunasin-based health-promoting products or drugs used in the treatment of cancer.

Molecular mechanisms of lunasin in the prevention and treatment of cancer. A previous study has demonstrated that lunasin is able to suppress the proliferation and migration of cancer cells caused by chemical carcinogens without any impact on wild-type cells (35). The potential underlying molecular mechanisms of lunasin in the prevention and treatment of cancer have been systematically investigated. Lunasin exhibits anti-mitotic effects, and may prevent the mutation caused by the chemical carcinogen 7,12-dimethylaminobenzaldehyde (DMAB) and viral oncogene early region 1A (E1A) in mammals (36). When DMAB or methylcholanthrene is present alone, lunasin at between 10 nmol/l and 10 μmol/l, was able to inhibit the mutation of cells by between 62 and 90% (36). In retinoblastoma (Rb) and DMAB-induced mouse embryonic fibroblasts, lunasin was able to inhibit the expression of oncogenes, the acetylation of histone H3 and the migration of tumor cells, and regulate the cell cycle to induce apoptosis, thereby inhibiting tumor formation (36,37). In human breast cancer MDA-MB-231 cells, lunasin was able to reduce the expression of cyclin D1/D3, and down-regulate protein kinase Bα(PKBα) and activator protein 1 (AP-1), thereby inhibiting cell proliferation and inducing apoptosis (38,39). In human leukemia L1210 cells, lunasin was able to reduce cell proliferation and induce apoptosis (40,41). Similarly, in DMAB-induced mouse skin tumors, lunasin was able to decrease the number of tumors generated and delay the generation of tumors in skin (42,43). Therefore, lunasin has been identified as a promising polypeptide for preventing tumor formation induced by chemical carcinogens.

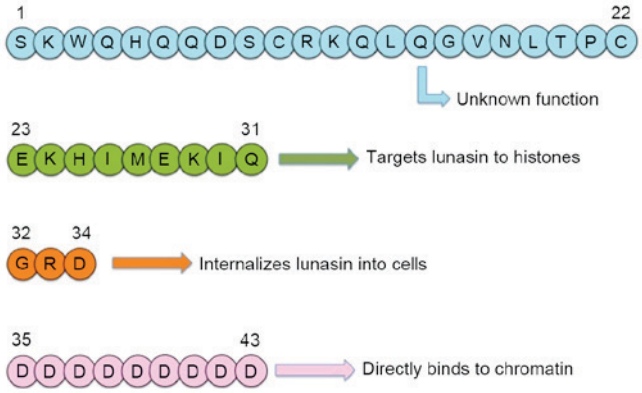

Figure 1. Sequence and structure of the fragments of lunasin. Figure previously published in (14).

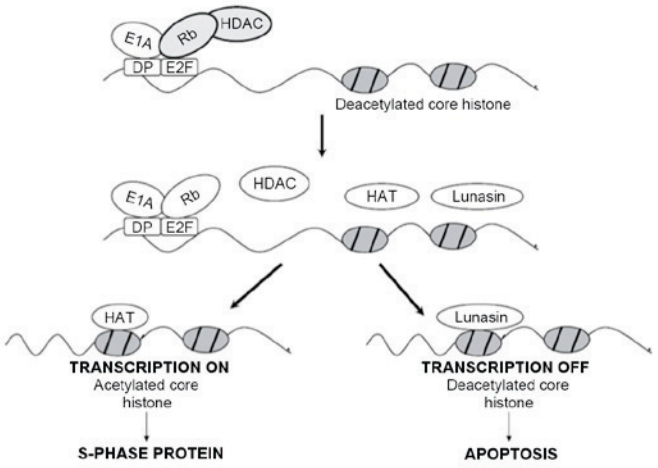

Figure 2. E1A-Rb-HDAC model describing the inhibition of E1A-induced cell transformation in the presence of lunasin. Figure previously published in (51). E1A, early region 1A; Rb, retinoblastoma; HDAC, histone deacetylase; DP, dimerization partner; E2F, transcription factor E2F; HAT, histone acetyltransferase.

In addition to the above-mentioned inhibition of cancer cell proliferation, epigenetic mechanisms have also been specifically analyzed during the application of lunasin. Acetylation is one of the most important modifications in cancer signaling pathways (44,45). DNA damage and the disruption of chromatin transcription demonstrate a marked association with the occurrence of acetylation of specific lysine residues on histones (46). Under normal circumstances, histones H3 and H4 are in a deacetylated, or suppressed state. Therefore, the decreased activity of histone acetyltransferases (HATs), including lysine acetyltransferase 2A/2B and p300, is consistent with the decreased incidence of cancer, including colorectal and breast cancer (47-49). In previous studies, lunasin was demonstrated to competitively inhibit HATs, thereby inhibiting acetylation and regulating the cell cycle (24,32). The underlying molecular mechanisms of the binding of lunasin to histones remain unclear; however, they may be associated with the helical structure formed by the amino acid residues at positions 23-31, and the specific spatial structure or orientation of lunasin (42). However, the inhibitory activity of histone acetylation was also markedly associated with the concentration of lunasin. Lunasin at a concentration of 10 nmol/l was able to decrease the acetylation of histones by between 20 and 25%; by contrast, lunasin at a concentration <1 μmol/l was able to decrease the acetylation of histones H3 and H4 by 80 and 74%, respectively (50). Lunasin was able to act on cells at the stage of division or transformation, which is described by the E1A-Rb-histone deacetylase (HDAC) model (Fig. 2) (51). The tumor suppressor protein Rb interacted with eukaryotic initiation factor to supplement HDAC and maintain the deacetylated state of core histones (52). E1A resulted in the inactivation of Rb, thus leading to the disassociation of the Rb-HDAC complex and the exposure of acetylated histones (52). Following acetylation of HATs, lunasin and HATs competitively deacetylate histones to terminate transcription, thus leading to apoptosis. Therefore, lunasin is involved in the destruction of acetylation-deacetylation kinetics of histones (32). Deacetylated histones are absent from wild-type cells, so lunasin was not able to disrupt the proliferation or cause the apoptosis of wild-type cells. While monitoring the cell cycle process in the presence of lunasin, lunasin was able to be activated to selectively kill tumor cells following the transformation of the cells from wild-type cells to tumor cells, and it was demonstrated that lunasin exhibits a limited number of side effects compared with other drugs (32,53).

Previous studies have demonstrated that chronic inflammation and oxygen free radicals are involved in degenerative diseases, inflammatory diseases, cardiovascular diseases and cancer (54,55). Therefore, anti-inflammation or antioxidation may be an important intervention strategy for the prevention and treatment of cancer (56). Lunasin was demonstrated to exhibit certain anti-inflammatory effects. i) Lunasin was able to inhibit the production of interleukin 6 (IL-6), tumor necrosis factor α and prostaglandin E_2 (PGE_2) in lipopolysaccharide (LPS)-induced RAW264.7 cells (57), and suppress the generation of IL-6 and IL-8, as well as suppress the secretion of matrix metalloproteinases in cultured rheumatoid arthritis synovial fibroblasts through the inhibition of nuclear factor kB activity (58). ii) Lunasin was also able to induce the inducible nitric oxide synthase/nitric oxide signaling pathway through adjustment of the cyclo-oxygenase 2/PGE_2 ratio (59). iii) Lunasin was able to perform its antioxidant function through decreasing LPS-induced reactive oxygen species production (57), and prevent the oxidative damage of DNA by inhibiting the generation of hydroxyl free radicals (60). The anti-inflammatory and antioxidant functions of lunasin improve its potential use in the prevention and treatment of cancer.

Synergistic prevention and treatment of cancer using lunasin with other anti-cancer compounds. In order to achieve a synergistic effect on the prevention and treatment of cancer, the combinatorial application of two or more drugs is a commonly used strategy, which is able to increase the treatment efficacy and decrease the toxicity of the drugs (6). In the past two decades, various studies have demonstrated that aspirin exhibits clear inhibitory activity in chemical-induced carcinogenesis (61,62). However, the side effects of aspirin, including peptic ulcers, mucosal injury and bleeding, are evident (63). In order to use aspirin for its anti-carcinogenic effects and reduce its side effects, a number of studies have

been conducted to explore the possibility of combinatorial application of aspirin and lunasin (37,39). As demonstrated by previous studies, the combinatorial application of aspirin and lunasin does not result in any safety issues (37,39). In addition, the combinatorial application of aspirin and lunasin resulted in a synergistic effect on the inhibition of proliferation and induction of apoptosis in human breast cancer MDA-MB-231 cells (39). This effect is primarily due to the down-regulation of PKBα, proto-oncogene Fos and AP-1 signaling genes through modulation of the genes that code for G_1 and S phase regulatory proteins, thereby achieving a marked decrease in the side effects of aspirin (39). Furthermore, similar results have been observed in DMAB-induced fibroblast tumors in mice (37). However, the potential advantage of increasing anti-cancer activity and decreasing the side effects requires further study in animals and humans, and the underlying molecular mechanisms for the synergistic effects of lunasin and aspirin on the prevention and treatment of cancer require further clarification. Such studies may be of benefit for the development of a novel combinatorial treatment of cancer.

5. Conclusions

Numerous studies have demonstrated that lunasin may serve a role in the prevention and treatment of various types of cancer. However, the underlying molecular mechanisms of the prevention and treatment of different types of cancer or cancer stages, and the enhanced bioavailability of lunasin during oral administration remain unclear, and require further investigation or clarification using genomic or proteomic methods. In addition, the optimal fragment length, secondary or tertiary structure, or specific spatial orientation of lunasin in the prevention and treatment of cancer requires clarification. Furthermore, lunasin primarily exists in soybean, which is a principal agricultural product in China. Due to the potential activity in the prevention and treatment of cancer, and the prevention of cardiovascular diseases and inflammatory diseases, polypeptides, including lunasin from soybean, require investigation for the development of medical or healthcare products.

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