Lunasin—a multifunctional anticancer peptide from soybean

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Scientific Note

Abstract

Lunasin is a bioactive peptide that was originally isolated from soybean and has since been shown to have a number of biological activities, including both cancer chemopreventive and therapeutic activities. Our recent focus has been on determining the range of cancer types that lunasin can affect and the mechanism of action against specific cancers. We recently found that lunasin has significant therapeutic activity against non-small cell lung cancer (NSCLC) both in vitro and in vivo. Mechanistic studies using lunasin-sensitive and lunasin-resistant NSCLC cell lines revealed the lunasin blocks cell proliferation by inhibiting cell cycle progression at the G1/S phase interface and that this inhibition was associated with reduced Akt signaling. In addition, we found that these effects were linked to the inhibition of integrin signaling through αv-containing integrins. Our results provide strong support for the hypothesis that direct effects on integrin signaling represent a major mode of action responsible for lunasin's anticancer activity.

Keywords: Lunasin, Bioactive peptide, Cancer Therapeutic, Integrins, Cell cycle, Non-small cell lung cancer

1. Introduction

Numerous studies over the years have found a strong linkage of high soy consumption with a number of health benefits, including lower rates of cancer. In recent years, it has become clear that at least part of the anticancer activity of soy is due the presence of the peptide-lunasin. Lunasin is a 43-44-amino acid peptide that is a component of the soybean 2S albumin protein that was initially shown to cause mitotic arrest and cell death in mammalian cancer cells.¹ Recent studies have now shown that lunasin has the capacity to inhibit the growth of many cancer cell types including breast cancer, colon cancer and lung cancer.² ³ ⁴ Thus, it is clear that lunasin may have potential as a therapeutic agent for the treatment of several deadly cancers.

Lunasin has three motifs that may be responsible for its biological activity against cancer; 1) a predicted helix domain homologous to a conserved region of chromatin-binding proteins, 2) a Arg-Gly-Asp (RGD) cell adhesion motif, and 3) a unique polyaspartic-acid tail (Figure 1). It was initially speculated that the RGD cell adhesion motif is involved in lunasin internalization into the cell, and that helix domain and poly-D tail is required for binding with core histone H3 and H4.⁵ Based on these hypotheses, the initial proposed mechanism for lunasin action was that lunasin competes with histone acetyltransferases by binding to deacetylated histones, resulting in an inhibition of histone acetylation and a concomitant down regulation of cell cycle-related protein expression and the activation of apoptosis. Based on these studies, my laboratory has focused on characterizing the effects of lunasin on lung cancer and elucidating its mechanism of action.

Figure 1: Amino-acid sequence and functional motifs of lunasin.

2. Results and Discussion

Our focus on lung cancer is based on the fact that it is the leading cause of cancer-related deaths among both men and women in the United States, and increasingly, around the world. Lung cancer is divided into two types, small cell lung cancer and non-small cell lung cancer (NSCLC); more than 80% of all incidences of lung cancer are NSCLC.² Our initial studies focused on assessing the...
effects of Lunasin on established NSCLC cell lines in vitro. These studies revealed that all the NSCLC lines tested were sensitive to lunasin; however, the surprising finding was that only one cell line, H661, was sensitive when assayed in standard adherent culture conditions. The other cell lines were only sensitive when assayed in non-adherent, anchorage independent conditions using a colony-formation assay. Our subsequent studies demonstrated that lunasin was also able to significantly inhibit tumor growth in a mouse xenograft model of NSCLC. Lunasin treatment (30 mg/kg body weight) reduced tumor size of subcutaneous tumors initiated by implanting NSCLC H1299 cells in nude mice by 63% (Figure 2).

These findings are very encouraging and showed for the first time that lunasin was active against NSCLC. Further studies on the molecular mechanism of lunasin-mediated inhibition of cell proliferation were done by comparing the responses of NSCLC cells under adherent conditions where line H611 is lunasin-sensitive and H1299 cells are resistant. These studies revealed that lunasin’s ability to inhibit H611 proliferation was due to the suppression of phosphorylation of the retinoblastoma protein and the concomitant inhibition of cell cycle progression at the G1/S phase transition. A summary of these results is depicted in Figure 3 which identifies key regulatory points where lunasin appears to have an effect.

As previously discussed, earlier reports showed lunasin inhibits histone acetyltransferases activity under in vitro condition, and it is well documented that epigenetic changes involving histone modifications are important in initiating and maintaining a cancer cell phenotype. Several studies have documented a direct interaction of lunasin with the core histones H3 and H4 in vitro, so we initiated experiments to see if we could detect interactions of H3 and H4 in cells. For this, proximity ligation assays (PLAs) were used to demonstrate lunasin interacts with histone in vivo using cell lines H661 and H1299 grown in adherent culture conditions. The lunasin-H3 interaction levels were significantly higher in lunasin-sensitive H661 cells compared to the lunasin-insensitive H1299 cells, whereas lunasin-H4 interaction levels were similar in both cell lines. These histone interactions were associated with inhibition of histone acetylation at H4K8 and H4K12 in the both cell lines (Figure 4). Interestingly, H661 exhibited increased histone acetylation level at H4K16 compared to H1299, suggesting a role for this histone acetylation mark in lunasin sensitivity. Further studies are required to functionally test whether lunasin-histone interactions are required for lunasin’s antiproliferative effects and the specific epigenetic changes associated with lunasin sensitivity.

Besides affecting histone acetylation, it is possible that lunasin could also affect integrin signaling through its RGD domain. Integrins are well known regulators of cell growth, migration, survival, and differentiation. Integrins are heterodimeric transmembrane receptor composed of two distinct α and β subunits. The integrin signaling cascade starts with activation by binding of various extracellular matrix proteins such as fibronectin, vitronectin and thrombospondin to the integrin extracellular domain. When integrins are inactive, the integrin β subunit cytoplasmic tail forms a salt bridge with the integrin α subunit tail. The binding
of extracellular ligands with the extracellular domain disrupts α and β subunit cytoplasmic tail associations, triggering binding of activation proteins such as kindlin to integrin β subunit tails and the initiation of further downstream signaling.18-23

We tested whether lunasin could affect integrin in two ways. First, we used PLAs to assess the ability of lunasin to interact with integrins, followed by investigations assessing lunasin effects on downstream signaling events. We found that lunasin interacted with integrin subunits α5 and αv in lunasin-sensitive H661 and lunasin-insensitive H1299 cells; however, the interaction level with integrin αv was significantly higher in H661. Based on the differential binding intensities of lunasin to different integrin subunits, we hypothesize that in H661 cells, lunasin suppresses cell proliferation through binding to integrin αvβ3. We confirmed that lunasin does indeed bind with αvβ3 using co-immunoprecipitation assays (Figure 5). Furthermore, lunasin treatment in H661 impaired binding of the direct effectors ILK, FAK and kindlin to integrin β1 and β3 cytoplasmic tails, which is the important initial step for activation of integrin signaling.24, 25 A similar disruption of direct effector interactions with integrins was not detected in the lunasin-insensitive H1299 cells. To functionally confirm that the effects of lunasin are mediated by an αv subunit-containing integrin, we used siRNA-mediated gene silencing to knock out expression of αv in H661 cells. Although H661 cells with silenced αv expression exhibited reduced proliferation in the absence of lunasin (thus verifying that αv is indeed a therapeutic target in this cell line), treatment with lunasin did not induce any further decrease in proliferation. This represents the first clear demonstration that lunasin’s ability to inhibit proliferation in NSCLC cells requires an αv-containing integrin.

To further assess lunasin effects on integrin signaling and extend our functional studies, we examined the activation of key integrin signaling components that are known to ultimately regulate cell proliferation using Western blot analyses. These studies revealed that lunasin treatment reduces integrin signal-regulated phosphorylation on FAK, Akt and ERK1/2 in H661 but not in H1299. Taken together, all of these results strongly suggest that in NSCLC cells, lunasin functions as an integrin-signaling antagonist to inhibit cell proliferation. Our current working model describing lunasin’s mechanism of action is shown in Figure 6.

Figure 5: Co-immunoprecipitation (IP) and western blot analyses of lunasin-αvβ3 interactions. NSCLC H661 cells were treated with 100 µM lunasin for 24 h. Cell lysates were prepared and immune-precipitates isolated using anti-lunasin or anti-αvβ3 antibodies. IPs were subjected to immunoblot analyses using the indicated probe antibodies.

Figure 6: Model for lunasin’s inhibition of cell cycle progression through suppression of integrin signaling. Red and green arrows indicate the activation or inhibition, respectively, of key regulatory proteins. Red X’s indicate disruption of key protein-protein interactions.
3. Conclusion

Lunasin is an intriguing multifunctional bioactive peptide that has significant potential to be developed into an anticancer therapeutic and/or chemoprevention agent. Our studies showed that lunasin has substantial anticancer activity against NSCLC cells both in vitro and in an in vivo mouse xenograft model. Extensive functional studies demonstrated that lunasin interacts with αv-containing integrins and likely functions as an integrin signaling antagonist. We have recently extended our studies into malignant melanoma and shown similar anticancer effects both in vitro and in vivo for this deadly cancer. Current studies are focused on the further development of lunasin as a therapeutic.

Conflict of interest

JI declares that he has no competing interests. KRD is listed as an inventor on two issued patents relating to the expression and purification of lunasin peptides and may benefit financially if the technologies described in these patents are licensed or sold.

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