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Current Opinion in Pharmacology

Development of the plant-derived peptide lunasin as an anticancer agent

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The health benefits of soy consumption have long been recognized. An important potential benefit is the linkage of soy consumption with reduced cancer risk. One emerging factor that may confer the anticancer effects of soy is the peptide lunasin. Lunasin has both chemopreventive and therapeutic activities against a variety of carcinogens and cancer types. A novel feature of lunasin is that it contains multiple functional domains that can modulate gene expression through effects on histone acetylation and integrin signaling. Recent studies suggest that lunasin effects on integrin signaling in cancer stem cells reduce expression of stemness factors with a concomitant reduction in metastatic potential. Here, we highlight recent studies of the potential use of lunasin as an anticancer agent and its mode of action.

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Current Opinion in Pharmacology 2018, **41**:27–33

This review comes from a themed issue on **Cancer**

Edited by **Elizabeth Yeh**

https://doi.org/10.1016/j.coph.2018.04.006

Introduction

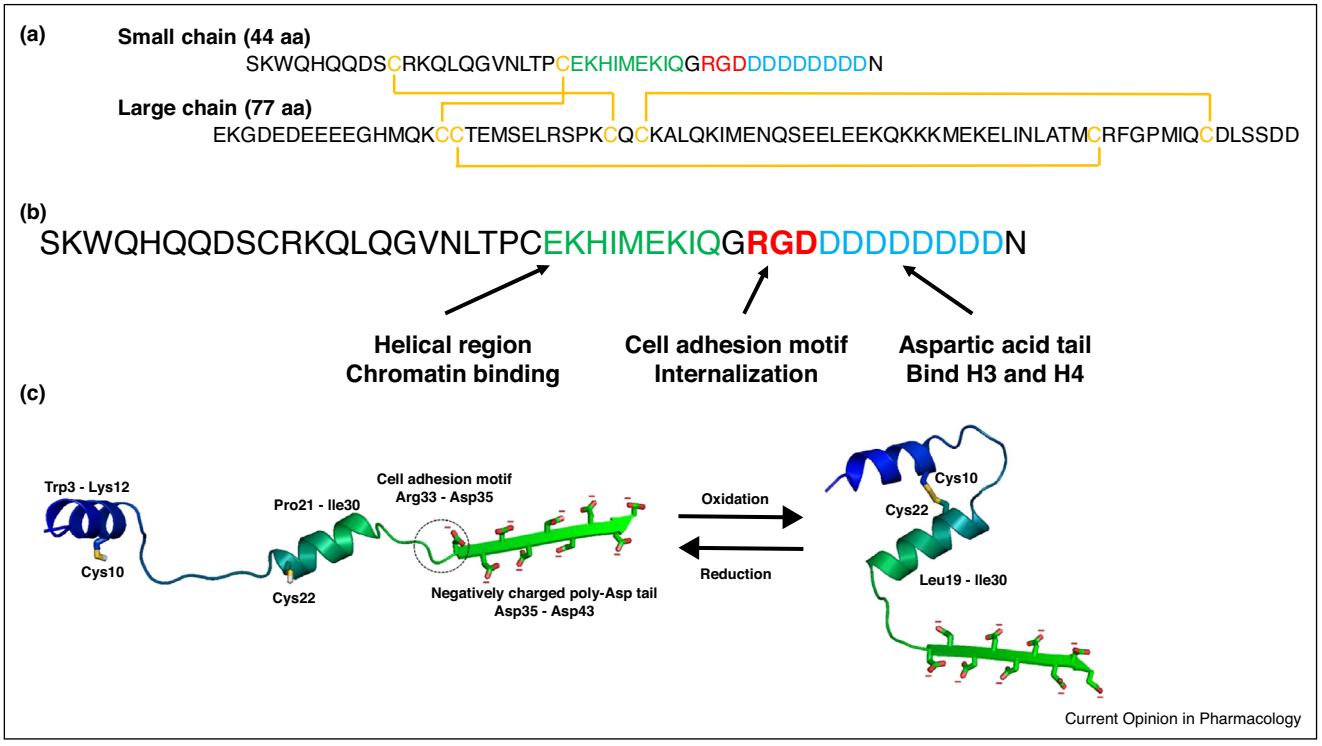
Plants have long been a source of medicinal compounds and major efforts have been focused on identifying plant-derived compounds for the treatment and prevention of cancer [1–3]. We have been intrigued by epidemiological studies that correlated high levels of soybean consumption with lowered incidence and mortality due to breast, prostate, colon and lung cancer [4–13]. The anticancer effects of soy components, particularly in breast cancer, have been attributed to secondary metabolites such as isoflavones [14–16]. Although there has been substantial effort to understand the potential anticancer effects of isoflavones, there has been little clinical success in translating this knowledge. However, it is acknowledged that not all of the anticancer effects associated with soy consumption are likely due to isoflavones [17•]. There is now extensive evidence that a significant component of the anticancer activity of soy is due to the presence of the bioactive peptide lunasin [18–22]. Lunasin is a 44 amino acid peptide component of the 2S albumin seed protein that has three putative functional domains: an aspartic acid tail, an RGD domain, and a chromatin-binding helical domain (Figure 1) [23•,24]. These domains all appear to be functional; the aspartic acid tail affects histone acetylation *via* interactions with core histones H3 and H4; the RGD domain mediates both internalization and modulation of integrin signaling; and the chromatin-binding domain provides an immunomodulatory function [25••,26,27,28,29,30••,31].

Notably, one of the first biological activities attributed to lunasin was its chemopreventive activity [32,33,34•]. Surprisingly, very little has been done to explore this important facet of lunasin biology. Early studies demonstrated that lunasin has the ability to suppress transformation of cultured mammalian cells by oncogenes (E1A-mediated and *Ras*-mediated) and chemical carcinogens 7,12-dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene (MCA), as well as DMBA-induced skin tumorigenesis in a mouse model [35–39]. A later study indicated that the ability of lunasin to inhibit foci formation in MCA and DMBA NIH/3T3 cells was enhanced when combined with aspirin [40]. Other than the DMBA-skin tumorigenesis study mentioned above, there is only one other *in vivo* chemoprevention study published. This study demonstrated that a 2-month pretreatment of nude mice with lunasin caused a 33-44% reduction in tumor incidence caused by MDA-MB-231 breast cancer cells [41]. In addition, lunasin was shown to be bioavailable and accumulated in numerous tissues, including the mammary gland. A preliminary study has indicated that dietary lunasin is bioavailable in humans [42], suggesting the potential for developing oral formulations that deliver efficacious levels of lunasin. These initial chemoprevention studies are promising and provide support for further studies on the potential use of lunasin as a chemoprevention agent.

Therapeutic activity of lunasin

Much of the recent research on lunasin has focused on its therapeutic activity. The cancer therapeutic activity of lunasin in both *in vitro* and *in vivo* models is quite broad and extends to lung cancer, colon cancer, leukemia,

Figure 1



Origin of lunasin in soybean. Lunasin is a component of the soybean 2S albumin seed protein encoded by the *GM2S-1* gene. The 2S albumin protein is produced as a preprotein that undergoes proteolytic processing to yield a mature protein composed of two subunits covalently linked through disulfide bridges. **(a)** Amino acid sequence of the large and small chains showing the intra-chain and inter-chain disulfulide bridges. The small chain represents lunasin. **(b)** The primary sequence of lunasin with the three putative functional domains indicated. **(c)** The proposed structure of lunasin. Lunasin has transient secondary structural elements that result in an intrinsically disorded peptide. Lunasin exists in either an oxidized or reduced form [62]. Used with permission from Elsevier.

melanoma, and breast cancer [25••,26,43,44,45•,46]. Mechanistic studies indicate that lunasin blocks the cell cycle, and in some cases induces apoptosis. There is some divergence in the cell cycle effects reported in different cancer cell types. Lunasin arrested the cell cycle at the G2/M phase in colon cancer cells and induced apoptosis in one study [47] whereas, another indicated a modest induction of apoptosis without any effect on cell cycle [45•]. These differences may be due to the fact that different cell lines were tested and that in one case [47], a purified lunasin (>90% purity) was used whereas the later study [45•] used synthetic lunasin. Lunasin also induced apoptosis in breast cancer cells *via* a PTEN-dependent mechanism [45•]. In non-small cell lung cancer (NSCLC) cells, lunasin caused arrest at the G1/S phase without apoptosis and was as associated with suppressed phosphorylation of the retinoblastoma protein [26].

In vivo murine models have validated the therapeutic effects of lunasin for several cancer types. Lunasin delivered by intraperitoneal injection at 30 mg/kg reduced tumor growth in NSCLC and melanoma xenografts by 63% and 55%, respectively [25••,26]. In a syngeneic model of melanoma, lunasin reduced tumor growth by 55%, indicating that lunasin is equally efficacious in both immunodeficient and immunocompentent murine models [48]. Importantly, the human melanoma xenograft studies demonstrated that daily injection of lunasin for 34 days did not induce any toxic effects [25••]. Recent studies demonstrating that lunasin can target putative cancer stem cells (CSCs) makes potential utility of lunasin as a therapeutic more compelling. Lunasin reduced the CSC populations of human melanoma and colon cancer cell lines *in vitro* [25••,45•], and reduced tumor growth 73% in xenografts initiated with human melanoma CSCs [25••]. Relevant to lunasin's effects on CSCs, several groups have now shown that lunasin suppresses metastasis and metastasis-related phenotypes. Lunasin inhibits cell migration *in vitro* and metastasis *in vivo* in models of colon cancer, melanoma and breast cancer [43,47,49•,50,51,52••]. Taken together, these results strongly suggest that lunasin attacks critical functions required for progression of multiple disparate cancer types.

Immune effects of lunasin

A potentially important and currently understudied aspect of lunasin's potential use in cancer therapy is its ability to activate innate immunity. One study has shown