

Understanding Lunasin's biology and potential as a cancer therapeutic by utilizing *Drosophila* genetics

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Abstract

Soy contains many bioactive molecules known to elicit anticancer effects. One such peptide, Lunasin, has been shown to selectively act on newly transformed cells while having no cytotoxic effect on non-tumorigenic or established cancer cell lines. While this effect on *in vitro* systems is promising, Lunasin's efficacy in an *in vivo* system is yet to be assessed. In this review, we discuss the state of knowledge with respect to Lunasin and then review some of the powerful genetic tools available in *Drosophila*. The availability of a sophisticated genetic tool box makes *Drosophila* an excellent genetic model well suited to studying the biology of Lunasin and its effect on tumor progression in an *in vivo* model organism.

Keywords: Lunasin, cancer, *Drosophila*, metastasis, tumors

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Introduction

History of soy

Soy (*Glycine max*) has been a staple in the diets of many cultures for millennia. Originally cultivated in Asia, soy arrived in Europe in the 1700s CE and by the 1800s was being grown in the United States. There are many health benefits associated with soy rich diets. Soy consumption lowers bad cholesterol, LDL,^{1,2} and reduces risk of coronary disease and cancers, particularly breast, colon, and prostate.³ Soybeans contain many bioactive compounds with demonstrated anticancer activity including isoflavones, protease inhibitors (most noted is the Bowman-Birk protease inhibitor, BBI), saponins, and more recently the peptide Lunasin.⁴

Lunasin was discovered by Odani *et al.* while isolating the 2S cDNA which codes for a nutrient enhancing, methionine-rich albumin protein in soy.⁵ The isolated gene, *Gm2S-1*, codes for the methionine-rich protein, and three additional peptides: a signal peptide, a linker peptide, and Lunasin (Figure 1) all of which are produced as a proprotein and then post-translationally processed.⁶ Lunasin has also been found in wheat,⁷ barley,⁸ amaranth,⁹ pepper,¹⁰ rye,¹¹ oats,¹² and the herb *Solanum nigrum*.¹³ As Lunasin has been isolated from a variety of angiosperm seeds, it is possible that a more thorough screening of plant seeds could reveal Lunasin from many additional sources. However, to date, soy remains the richest source of Lunasin.

Structure of Lunasin

Lunasin is a 43 amino acid peptide, which is organized into four motifs; an N-terminal region of unknown function followed by a predicted helical region, a carboxyl terminal RGD cell adhesion motif, and a run of eight aspartic acid residues (poly-D tail) (Figure 2).¹⁴ The helical region is homologous to chromatin binding proteins. The N-terminus, predicted helix, and the poly-D tail are essential for the bioactivity of Lunasin; however, there is conflicting evidence as to whether the RGD motif is essential for the anticancer effects (Table 1).^{14,15} The poly-D tail binds to deacetylated histones. Galvez and de Lumen found that the poly-D tail and RGD motif are necessary for the antimitotic effect of Lunasin. This was demonstrated in both murine hepatoma cells and *Escherichia coli* (*E. coli*) cells.¹⁴ Transfection of murine hepatoma cells with full-length *lunasin* resulted in a diffused chromosomal mass, indicative of mitotic disruption, whereas transfection with a poly-D deletion mutant resulted in a loss of antimitotic activity (i.e. chromosomal DNA appeared normal and there were no morphological changes). This poly-D deletion also disrupts the RGD motif and so it is not possible to distinguish whether the loss of antimitotic activity is a result of poly-D deletion or RGD disruption. Moreover, *E. coli* cells expressing full-length *lunasin* exhibited reduced bacterial growth and abnormal filament formation. On the other hand, *E. coli* cells expressing a *lunasin* deletion mutant (resulting in

inability of Lunasin to be internalized by cells and thus it was concluded that the RGD motif is necessary for internalization of Lunasin.¹⁶ In a later study by Lam *et al.*, Lunasin lacking the RGD motif was only slightly less effective at preventing transformation of mouse embryonic fibroblasts (NIH3T3) compared with full length Lunasin. Additionally, they reported that RGD-deletion mutants are internalized into the nucleus as effectively as full-length Lunasin, demonstrating that the RGD motif is not required for internalization.¹⁵ Lam *et al.* propose that this discrepancy in the data is due to differences in internalization mechanisms between cell lines (NIH3T3 and C3H 10T1/2).

Natural role in soy

Lunasin is found in abundance in developing soy cotyledons. There are three stages in the development of the cotyledon: (1) a phase of rapid cell division followed by (2) a cessation of mitosis, during which time cells grow larger and acquire and store nutrients, and (3) dehydration of the seed. The expression of Lunasin coincides with mitotic arrest. It is thought that Lunasin may play a role in triggering the arrest of cell division because of its demonstrated antimitotic activity *in vitro*.¹⁷

Effects in tumorigenic and non-tumorigenic lines

Lunasin exhibits no cytotoxic or antimitotic effects on non-tumorigenic or established cancer cell lines.^{15,16,18,19} When treated or transfected with Lunasin, these cells show normal growth and cell division. In contrast, in cells newly transformed by chemical carcinogens or viral oncogenes, Lunasin has been shown to suppress transformation, arrest cell division, and induce chromosomal fragmentation and apoptosis.^{15,16,18,19}

Proposed mechanisms of action

Inhibition of core histone acetylation

Under non-mitotic, steady-state conditions, core histones are typically in a repressed, deacetylated state. During a transformation event the histones are acetylated, which relaxes the chromatin, and results in the initiation of transcription.¹⁶ Interruption of acetylation is perceived by the cell as abnormal, and in response the cell commits itself to apoptosis. Lunasin competes with histone acetyltransferases (HATs) to bind the histones that are exposed during transformation events.¹⁶ If HAT binds, transcription is initiated, S phase proteins are produced (like DNA polymerase α and proteins of the origin recognition complex), and mitosis progresses. However, binding of Lunasin to exposed, deacetylated histones disrupts the acetylation process. This results in commitment of the newly transformed cells to apoptosis, thereby preventing the replication of neoplastic cells.²⁰ In this way, de Lumen *et al.* liken Lunasin to a "watchdog", whereby Lunasin sits ideally in the nucleus waiting for a transformation event to occur before jumping to action, inhibiting the replication of newly transformed cells.²⁰

Inhibition of mitosis by increasing expression of CDK-inhibitors, p21 and p27

Cyclin-dependent kinases (CDKs) are important in controlling cell cycle progression.²¹ Deregulation of the cell cycle is attributed to low levels of CDK inhibitors, such as p21 and p27, and is a hallmark of transformed cells.²² Additionally, low levels of p27 are correlated with a poor prognosis in breast cancer patients.²³ Lunasin has been shown to increase the expression of p21 and p27 (Figure 3), resulting in an arrest at the G2/M stage in transformed colon cancer cells (KM12L4) and mouse embryonic fibroblasts (NIH3T3).^{15,18}

Additionally, earlier work has shown that a reduction in p21 and p27 gene expression correlated with an increase in drug resistance.^{24,25} It is possible that the increased levels of these CDK inhibitors, as induced by Lunasin, will reduce the prevalence and slow the rise of drug resistance in patients.

Decreased metastasis via modification of ECM gene expression

Angiogenesis and metastasis are often preceded by deregulation and degradation of the extracellular matrix (ECM). Lunasin has been found to modify the expression of numerous genes involved in cell-adhesion and ECM regulation (Table 2).¹⁸ Matrix metalloprotease 10 (MMP10) has been shown to be a critical gene for progression of lung cancers²⁶ and in the presence of Lunasin, MMP10 is considerably downregulated (7.71 fold).¹⁸ Additionally, Lunasin upregulated the expression of COL14A1 (collagen, type XIV, $\alpha 1$), a protein that, when knocked down, results in increased cell proliferation in renal cell carcinoma cell lines.¹⁸

$\alpha 5\beta 1$ integrin plays a key role in cell adhesion, growth, and proliferation.²⁷ Elevated levels of $\alpha 5\beta 1$ are associated with increased invasiveness of colo-rectal cancer (CRC) cell lines²⁸ and loss of $\alpha 5\beta 1$ function results in decreased metastasis in CRC cells.²⁹ Lunasin is able to downregulate the expression of $\alpha 5$ integrin and has the most potent antimitotic effect on cell lines which highly express the $\alpha 5\beta 1$ integrin,¹⁸ suggesting a role as an anti-metastatic molecule.

Induction of apoptosis by activating the mitochondrial apoptosis pathway

Mitochondrial-mediated apoptosis can be triggered by a change in membrane permeability, which is regulated by proteins in the Bcl-2 super family and cytochrome c.³⁰ Lunasin has been found to activate the mitochondrial apoptosis pathway in colon cancer cells (KM12L4) by increasing levels of pro-apoptotic molecules such as Bax, nuclear clusterin, and caspases 2, 3, and 9, while reducing the expression of Bcl-2, a pro-survival molecule.¹⁸

Reduction of inflammation by inhibition of proinflammatory cytokines

Chronic inflammation has long been implicated as an initiating factor in many cancers (colon, liver, bladder, and stomach).³¹ During times of inflammation, leukocytes induce DNA damage through the production of genotoxins



Figure 3 Known modes of action of Lunasin. Lunasin brings about its anticancer effects in a variety of ways. The effect of Lunasin on cancer cells is shown in white lettering within the circles. The mechanism through which Lunasin exerts a specific effect is denoted in yellow. (A color version of this figure is available in the online journal.)

Table 2 Effects of Lunasin on gene expression in human KM12L4 colon cancer (adapted from (Dia and Gonzalez de Mejia¹⁸)

Functional gene groupings	Gene	Description	Fold change
Transmembrane molecules	ICAM1	Intracellular adhesion molecule, CD54	3.14
	ITGA5	Integrin, α5	−5.01
	ITGB1	Integrin, β1	1.38
	ITGB2	Integrin, β2	−8.19
Cell–cell adhesion	COL14A1	Collagen, type XIV, α1	11.62
Other adhesion molecules	COL6A1	Collagen, type VI, α1	−10.10
	LAMB3	Laminin, β3	2.09
	THBS1	Thrombospondin 1	−2.61
Extracellular matrix proteases	ADAMTS8	ADAM metallopeptidase with thrombospondin type 1 motif, 8	2.56
	MMP1	Matrix metallopeptidase 1	2.48
	MMP9	Matrix metallopeptidase 9	3.71
	MMP10	Matrix metallopeptidase 10	−7.71
	MMP11	Matrix metallopeptidase 11	2.33

including reactive oxygen species (ROS) and nitric oxide (NO). Recently, it has been suggested that Lunasin may help reduce inflammation by inhibiting the release of NO and pro-inflammatory cytokines like TNF-α and IL-1β.³ The discovery of the anti-inflammatory effects attributed to Lunasin may be significant in the search for therapeutics for other ailments directly caused or amplified by

inflammation including arthritis, heart disease, diabetes, and Alzheimer’s disease.

Upregulation of a tumor suppressor, PTEN

PTEN, a phosphatase and tensin homolog, is a tumor suppressor that plays a regulatory role in cell cycle progression

Table 3 Selected genetic tools available in *Drosophila*

Tool	Function	Utility in studying Lunasin	For additional information
Balancer Chromosomes	(1) Suppress crossover events during meiosis; (2) Allow for the maintenance of recessive lethal mutations in a stock	Create balanced stocks containing UAS- <i>lunasin</i> insertions	Roberts ⁴³
P-elements	(1) Induce mutagenesis; (2) Create transgenic/genetically modified lines	Create transgenic <i>Drosophila</i> containing <i>lunasin</i> gene	Ryder and Russell ⁴⁴
FLP/FRT	Induce site-specific recombination	Create clones homozygous for mutant tumor suppressor, <i>lgl</i>	Golic and Lindquist ⁴⁵
GAL4/UAS	Drive tissue specific overexpression of target gene	Study how expression of Lunasin affects normal development in <i>Drosophila</i>	Duffy ⁴⁶
MARCM	Create fluorescently labeled clones, homozygous for mutation of interest and overexpressing a gene of interest	Genetically create labeled tumors to study how Lunasin affects tumorigenesis	Wu and Luo ⁴⁷

and apoptosis, and is often found mutated or downregulated in cancers.^{32,33} Genistein, a soy isoflavone, has been found to impart antitumor effects via activation of PTEN pathways,^{34,35} leading researchers to wonder if Lunasin shares this antitumor mechanism. Pabona *et al.* found that human breast cancer cells (MCF-7) treated with Lunasin exhibited elevated levels of PTEN expression and nuclear localization resulting in the induction of apoptosis via the PTEN pathway.¹⁹

Most of the studies reviewed in this article have utilized *in vitro* systems to study the effects of Lunasin, with no studies addressing the effects of *in vivo* expression of *lunasin* on tumor growth and metastasis in an intact organism. In order for Lunasin to progress to advanced stages of drug development, it is imperative that the *in vivo* effects of Lunasin expression be assessed in a living organism. *Drosophila* provides a powerful, economical choice for a model organism in which to test this. Here we present reasons why *Drosophila* is a good choice for studying the *in vivo* effects of Lunasin and provide an experimental strategy for how this can be achieved in *Drosophila*.

***Drosophila* as a model organism**

Since the time of Thomas Hunt Morgan, *Drosophila* has been an important model organism. Morgan originally chose the fruit fly because of the ease of collection, fast generation time, and small size. Since his time, much information has been collected that leads researchers to continue to choose *Drosophila* as a model organism.

The power of *Drosophila* as a model lies in the simplicity of conducting genetic screens made possible by the ease and affordability of rearing multiple generations in a short period of time. *Drosophila* has an 8–10 day life cycle at 25°C with females laying between 500 and 1000 eggs per animal.³⁶ Vast quantities of eggs allow researchers to analyse hundreds or even thousands of mutant lines over multiple generations, a feat matched only by bacteria and yeast.³⁷

The *Drosophila* genome is organized into four chromosomes, containing approximately 13,600 genes³⁸; a relatively low number as compared to the estimated 20–30,000 genes in humans.³⁹ Furthermore, *Drosophila* has relatively little genetic redundancy as compared to vertebrate models³⁷ allowing for easier genetic mapping and balancing (discussed below). Additionally, the low number of orthologs present in flies for human genes presents a unique opportunity and an ease in understanding the gene function in an intact organism. For example, the mammalian genome contains more than 20 MMPs, whereas flies have only two MMPs.⁴⁰ To study the effect of total MMP knockdown it is easier to knock down the two MMPs in *Drosophila* than to mutate or knockdown the more than 20 MMPs in mammals.

Perhaps the most important aspect of a model organism is the ability to translate information gained to other organisms. As many processes and biochemical pathways are conserved from flies to humans,³⁷ *Drosophila* provides an informative model with which to study human disease states. About 80% of human genes have homologs in *Drosophila* and about the same percentage of identified human disease genes have *Drosophila* cognates (75%).^{41,42}

Availability of a sophisticated tool box in *Drosophila*

The expansive ‘tool box’ that exists for *Drosophila* research is rivaled only by bacteria and yeast, making fruit flies a very attractive genetic model. A listing of selected techniques used by the *Drosophila* community is presented in Table 3.^{43–47} The iconic tool used by *Drosophila* geneticists are the phenotypic mutations (e.g. red eye, *w*⁺) called ‘markers’. These mutations allow researchers to follow specific genes as well as whole chromosomes through multiple generations. The use of these ‘marker’ mutations, in combination with chromosomal inversions, has led to the development of another powerful tool, the balancer chromosome.

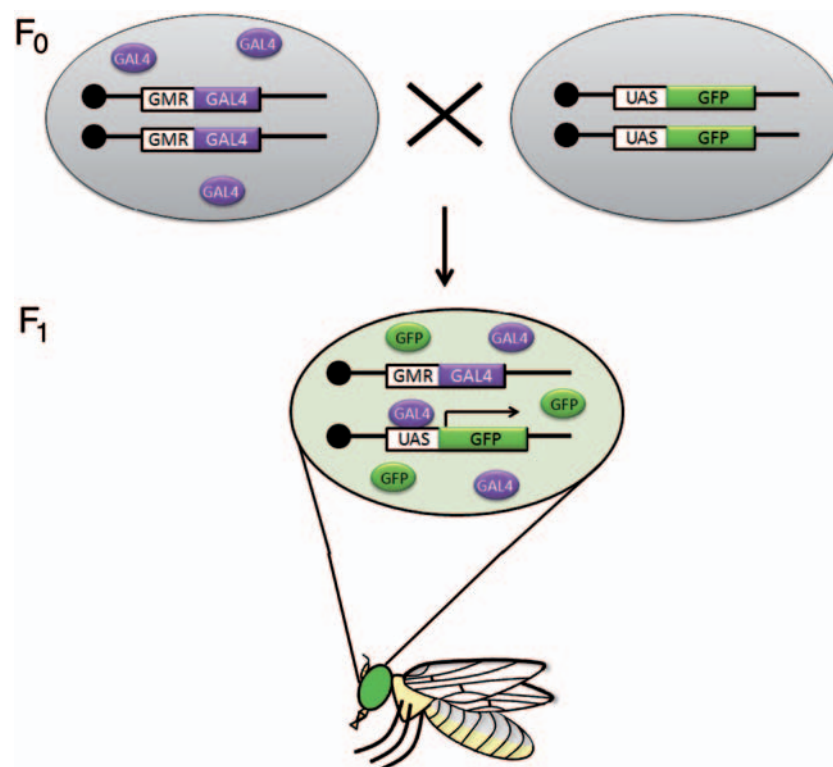


Figure 4 The *Drosophila* GAL4/UAS binary overexpression system for targeted gene expression. Expression of a gene of interest (in this case green fluorescent protein, GFP) in a targeted manner is brought about by crossing flies bearing a UAS-GFP transgene with flies containing a GAL4 driver under the control of an enhancer element (in this example the GMR enhancer). Progeny flies express GAL4 only where the driver, GMR enhancer, is active in the developing eye. GAL4 will bind to UAS and force the expression of the target gene, GFP, resulting in *Drosophila* with green fluorescing eyes. (A color version of this figure is available in the online journal.)

Balancer chromosomes are chromosomes that contain multiple inversions capable of suppressing crossover events between homologous chromosomes during meiosis. These chromosomes are marked by dominant, homozygous lethal mutations (e.g. Tb, Sb, ScO, etc.) and allow investigators to (1) maintain recessive lethal mutations in a population and (2) to know the genotype of individuals without having to genotype each animal.⁴⁸ Balancer chromosomes are available for the X chromosome, as well as all three *Drosophila* autosomes.⁴⁹

Another important advance in fly biology happened with the identification and engineering of P transposable elements (P-element). The re-engineered P-element and its incorporation into DNA vectors like pCASPER made it possible for researchers to create transgenics and insertional mutations for and in various genes, respectively.⁵⁰ Furthermore, the P-element also facilitated the development of enhancer traps and other genetic tools used by the fly community today.⁵¹

The GAL4/UAS system is one such tool in which the UAS and GAL4 elements are derived from yeast and, in combination with the P-element, results in a powerful gene expression technique. This technique is routinely utilized to achieve targeted overexpression of a gene of interest.⁵² To achieve this targeted overexpression, the gene is placed under the control of an upstream activation sequence (UAS) enhancer element which is responsive to the

transcription factor GAL4, and drives expression of downstream genes (Figure 4). GAL4 expression can be spatially restricted by placing GAL4 under the control of a tissue specific enhancer (e.g. the glass multiple reporter enhancer in GMR-GAL4 drives GAL4 expression to the eye). By forcing the ectopic expression of a gene, it is possible to study the biology of an otherwise lethal gene *in vivo*⁵³ in various tissue contexts. A number of GAL4 driver lines are available from the Bloomington *Drosophila* Stock Center (<http://flystocks.bio.indiana.edu/>) that allow for the expression of GAL4 in specific tissues.

Genetic recombination between homologous chromosomes is typically not found in somatic cells but is restricted to meiosis. Thus a recessive lethal mutation cannot be studied in its homozygous state in an individual fly as this would lead to lethality. The flippase/flippase recombination target (FLP/FRT) technique circumvents this problem and allows for mitotic recombination between homologous chromosomes in *Drosophila*. The resulting products of mitosis consist of clones of cells bearing the mutation in a heterozygous state, clones of cells bearing the mutation in a homozygous state and clones of cells homozygous for the wild type allele. The creation of clones that carry the mutation in the homozygous state allows for its study without outright killing the individual fly. By placing this recombination system under the control of a heat shock promoter or other tissue specific enhancers, we can temporally and

Table 4 Mammalian homologs to *Drosophila* genes related to tumorigenesis

Functional gene grouping	Fly gene	Homologous mammalian gene (or gene product)	Source
Oncogene	<i>D.Jun</i>	c-jun	Zhang et al. ⁶¹
	<i>aurora</i>	<i>aurora 1, aurora 2</i>	Bischoff et al. ⁶²
	<i>Notch</i>	<i>TAN-1</i>	Aster et al. ⁶³
	<i>D.int-1</i>	<i>int-1</i>	Rijsewijk et al. ⁶⁴
	<i>Ras</i>	<i>Ras</i>	Potter et al. ⁵³
	<i>hopscotch</i>	JAK kinase	Zeidler et al. ⁶⁵
	<i>stat92E</i>	STAT	Zeidler et al. ⁶⁵
	<i>hippo</i>	<i>MST1, MST2</i>	Polesello et al. ⁶⁶
Tumor suppressor	<i>Bunched</i>	<i>TSC-22</i>	Wu et al. ⁶⁷
	<i>Discs-large (Dlg)</i>	<i>Dlg-1</i>	Frese et al. ⁶⁸
	<i>D.PTEN</i>	<i>PTEN</i>	Potter et al. ⁵³
	<i>lats</i>	<i>LATS1</i>	Tao et al. ⁶⁹
	<i>patched</i>	<i>ptch</i>	Potter et al. ⁵³
	<i>lethal giant larvae(lgl)</i>	<i>lgl1, lgl2</i>	Humbert et al. ⁷⁰
Proto oncogene	<i>Wingless</i>	<i>Wnt-1</i>	Smolich et al. ⁷¹
	<i>awd</i>	<i>Nm23</i>	Potter et al. ⁵³
	<i>Hedgehog</i>	<i>vhh-1</i>	Roelink et al. ⁷⁷

spatially control mitotic recombination⁴⁵ and clone generation in the fly.

Another powerful technique emerged when Liquan Luo's group at Stanford combined the GAL4/UAS system with the FLP/FRT technique to develop the MARCM system short for 'mosaic analysis with a repressible marker'.⁴⁷ MARCM allowed researchers to create discrete, fluorescently labeled clones that are homozygous for a target gene, in an otherwise heterozygous animal.⁵⁴ Due to the presence of the GAL4/UAS system in MARCM, one can also simultaneously overexpress a gene of interest. These properties of the MARCM system have been utilized to model tumor progression and to study the tumor micro-environment interaction.^{55,56}

Biomedical advances attributed to fruit fly research

Drosophila has been used to model a variety of diseases and genetic disorders associated with aging,⁵⁷ neurological diseases such as Alzheimer's and Parkinson's,^{37,58} and cancers. *Drosophila* thus provides a valuable model to understand disease states in humans and has led to many advances in modern medicine.

One such advancement in medicine is the approval of a therapeutic for the treatment of tuberous sclerosis (TS). In patients with TS, tumors develop in multiple organs. Research on *Drosophila* elucidated the signaling pathway responsible for the misregulation of tissue growth, and furthermore led to the discovery that reducing the expression of a gene (S6 kinase) prevents this misregulation.⁵⁹ Rapamycin is a drug known to inhibit a portion of the S6 kinase pathway, and was approved by the FDA for clinical trials in TS patients. In 2008, Bissler *et al.* published findings that a 12-month regimen of rapamycin resulted in a decrease in angiomyolipomas in 50% of patients.⁶⁰

Modeling of tumors in *Drosophila*

Many *Drosophila* genes have homologs to human oncogenes and tumor suppressors making *Drosophila* a powerful/ useful model to study tumorigenesis and metastasis (Table 4).^{53,61-71} Research on *Drosophila* has led to the elucidation of a number of pathways that directly regulate tumor development in humans including the Notch, Hippo, JAK-STAT, LATS, and Hedgehog signaling pathways.^{53,55,72,73} The notch signaling cascade, which has been implicated in several human cancers including breast, ovarian, and skin cancers, was first identified in *Drosophila*.^{55,72} Additionally, genetic screens in *Drosophila* led to the identification and characterization of three key genes involved in the Hedgehog pathway, a major regulator of tissue growth which is linked to the transformation of healthy tissue to tumors in several cancers.^{37,73} While studies of cell cultures provide invaluable information, *Drosophila* affords us the opportunity to study the microenvironments of tumors *in situ*. Using the vast toolbox, we can readily manipulate genes *in vivo* and visualize the effects on tumor growth and metastasis.

Modeling of metastasis in *Drosophila*

The majority of cancer fatalities in humans are attributed to secondary metastatic growth.⁷⁴ As many biochemical mechanisms and pathways associated with metastasis are conserved between humans and flies (e.g. ECM turnover),⁵³ *Drosophila* provides a useful model for studying metastasis.

Features of tumor metastasis have been modeled in *Drosophila*. This modeling was achieved by utilizing the property of cooperation between an activated oncogene and loss of cell polarity function. Cooperation between these two functions results in massive overgrowth and

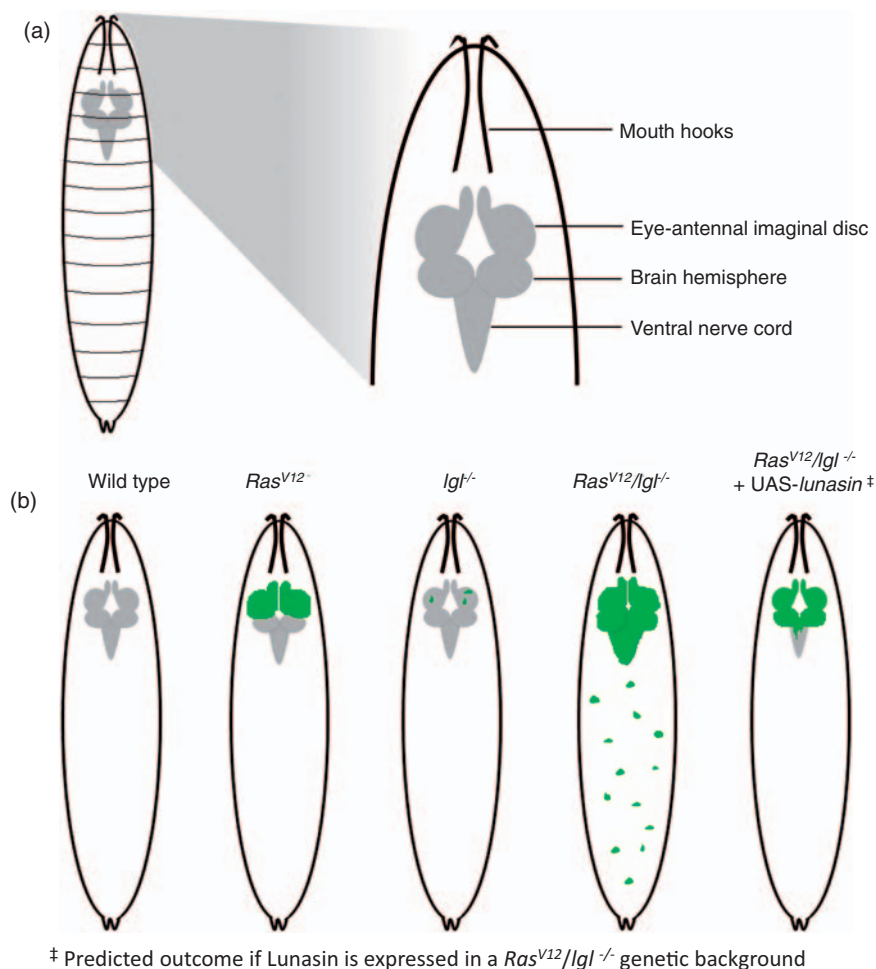


Figure 5 Modeling of benign and invasive tumors in *Drosophila*. (a) Drawing of a third instar larva is shown with an approximate location of the cephalic complex. A magnified view of the cephalic complex is presented on the right and various imaginal tissues are labeled. (b) Third instar larval schematics with genotypes utilized in modeling benign and invasive tumors are shown. Cephalic complexes are shaded gray except where tumor tissues marked green are present. No tumor tissue is present in wild type larva (first from left). Larvae overexpressing an oncogenic form of Ras, Ras^{V12} results in benign overgrowth in the eye-antennal disc as shown (second from left). Larval eye antennal disc cells mutant for *lgl* result in small clones (third from left). Cells overexpressing Ras^{V12} and simultaneously mutant for *lgl* result in overproliferation of eye-antennal disc cells (fourth from left). These cells are capable of invading other organs and display hallmarks of metastasis found in humans. The potential of Lunasin as an anticancer agent can be tested in these benign and invasive tumor cells by simultaneous overexpression from a UAS-*lunasin* transgene (fifth from left). In all panels tumor tissue is marked with GFP. The tumors were induced using the MARCM technique (see text for details). (A color version of this figure is available in the online journal.)

migration of tumors. One such modeling makes use of the $Ras^{V12}/lgl^{-/-}$ genotype.^{56,75} Ras is an oncogenic protein and Ras^{V12} codes for an activated isoform of the protein. Flies overexpressing Ras^{V12} exhibit benign, non-metastatic tumors. However, when Ras^{V12} clones are also mutant for lethal giant larvae, a tumor suppressor encoded by the gene *lgl*, aggressive metastatic tumors result.^{56,75}

Limitations of *Drosophila* as a tumor model

Although there are structures in flies that perform similar functions to mammalian organs (e.g. *Drosophila* Malpighian tubules and mammalian kidneys⁷⁶), several human organs have no direct homologous organ in flies. This can make modeling of organ specific cancers difficult. Regardless, there is much to be learned about the highly conserved biochemical characteristics of tumors that can be studied by mimicking tumor environments in the fly.

Instead of blood vessels, *Drosophila* utilizes an open circulatory system. Hemolymph serves as the nutrient transport system and the hemocoel, an open cavity, houses and transports hemolymph. Although true angiogenesis does not occur in *Drosophila*, in tumors produced by mutations in the *lats* gene, 'lumen like' structures, resembling blood vessels, form and serve to distribute nutrients,⁵³ mimicking angiogenesis. In this way, it is possible to study angiogenesis in flies as it relates to tumors; however, care must be taken to ensure conclusions are not overstated.

Use of *Drosophila* to study the effects of Lunasin *in vivo*

Lunasin is currently a focus of investigation for its role as a potential anticancer therapeutic. Many studies have investigated the effects of Lunasin *in vitro*, but to date no studies address the effects of *in vivo* expression of *lunasin*.

Drosophila provides a valuable tool in which to study the effects of Lunasin on tumor growth and progression *in vivo*. Transgenic *Drosophila* capable of expressing Lunasin under the control of the GAL4/UAS overexpression system in combination with *Ras*^{V12} benign or *Ras*^{V12}/*lgl*^{-/-} genetically induced invasive tumors can afford us the opportunity to assess how Lunasin affects tumor growth and progression in an *in vivo* model (Figure 5).

Concluding remarks: Lunasin as a natural chemotherapeutic

Aside from the demonstrated benefits of Lunasin, public opinion may very well play a role in the development of Lunasin as a therapeutic. Our society increasingly places a high value on natural products, as manmade chemicals often have unforeseen side-effects. It therefore makes sense for us to find ways to reduce our dependence on these chemicals. Because of the multitude of anticancer pathways influenced by Lunasin, this short soy bean derived peptide holds promise in combating numerous cancers, including those which have developed drug resistance. The next stage in Lunasin research is to test the efficacy of Lunasin *in vivo*, and *Drosophila* provides us a powerful model with which to do so.

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