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Abstract:
We propose to combine our expertise to target a process that is critical to breast cancer metastasis that is likely conserved in flies, mice and humans. The advantages of addressing the question of metastasis through the combined expertise of the Cagan and Weilbaecher labs is that we will use the powerful genetic tools provided by Drosophila that will identify key genetic pathways critical to tumor cell migration and metastasis that can be rapidly and rigorously tested. This a real time, in vivo dynamic screen that occurs in a whole organism. Tumors develop in the epithelial layer of the wing and the genetics of tumor cell invasion and migration throughout the organism can be modeled in real time, and genetically manipulated in large scale genetic screens. Dr. Weilbaecher’s laboratory will take advantage of the genetic knowledge gained from the Drosophila metastasis models in the development of an improved breast cancer metastasis mouse model. Dr. Cagan’s laboratory will be provided with mammalian human and murine breast cancers to validate their genetic and pharmacologic anti-metastasis strategies. Jointly, Drs. Cagan and Weilbaecher propose to develop novel therapeutics targeted to the metastatic process. In year one, we have identified 6 compounds that decrease metastasis in Drosophila metastasis model and decrease viability of mammalian breast cancer cells in vitro. We have validated the compound Cyclopamine, a hedgehog inhibitor, to block lung metastases in murine breast cancer xenograft and will use this as a template for testing other candidate therapeutic compounds from fly to mouse. Finally, we have uncovered a previously unknown and important connection between Src and Hedgehog signaling in mediating metastasis.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>3</td>
</tr>
<tr>
<td>Body</td>
<td>3-5</td>
</tr>
<tr>
<td>Key Research Accomplishments</td>
<td>5</td>
</tr>
<tr>
<td>Reportable Outcomes</td>
<td>5</td>
</tr>
<tr>
<td>Conclusion</td>
<td>5-6</td>
</tr>
<tr>
<td>References</td>
<td>6</td>
</tr>
<tr>
<td>Appendices</td>
<td>6</td>
</tr>
</tbody>
</table>
INTRODUCTION:
In this Synergy award, we proposed to bring compounds identified with a novel screening method to standard mouse breast cancer assays. We developed Csk/Src Drosophila models to explore specific aspects of overgrowth and metastasis. Both Src and the Csk paralog Chk have been implicated in breast cancer metastasis. We propose to test the hypothesis that drugs identified in our novel Drosophila wing model of tumor (invasive proliferation) and metastasis—targeting the effects of activated Src—will show efficacy in a mouse model of breast cancer and metastasis. The overall goal of this proposal is to validate the findings from a Drosophila metastasis model in murine and human breast cancers. Specifically, we will examine the interactions of epithelial tumor cells with bordering non-malignant epithelial cells, and whether these interactions alter the metastatic potential of cells at the tumor boundary. Several critical signaling pathways specific to this interaction have been identified in a Drosophila whole animal genetic screen. We propose to validate these pathways in mouse and human breast cancers, and to extend the Drosophila search for new factors. The long term goals of this proposal will be to identify critical targets involved in tumor progression and breast cancer metastasis using the power of forward genetics in Drosophila, and to develop novel murine breast cancer models of metastasis that can be used to screen new genes and therapeutics targeted to breast cancer metastasis.

BODY:
Our efforts were strongly successful, and we have a joint manuscript under review that presents our work as a new approach to breast cancer therapeutics:


The Cagan laboratory developed Csk/Src Drosophila models to explore specific aspects of overgrowth and metastasis. Both Src and the Csk paralog Chk have been implicated in breast cancer metastasis. We used these dCsk/Src metastasis models to identify candidate compounds that reduce the oncogenic-like effects of activated Src. Specifically, we utilized a model that contained ‘crumpled wing’ and ‘overgrown eye’ phenotypes due to reducing dCsk activity in the eye and wing (eye/wing>dCskRNAi; Figure). The precise genotype was GMR-GAL4/FM6, MS1096-GAL4 UAS-dCskRNAi/MS1096-GAL4 UAS-dCskRNAi. We screened the National Cancer Institute “Diversity Set” of 1990 compounds for chemicals that suppressed the eye/wing>dCskRNAi phenotype. The Diversity Set contains an eclectic collection of compounds that emphasize cancer-related compounds. Compounds scored as suppressing the eye/wing>dCskRNAi phenotype were confirmed in at least two additional re-tests.

356 compounds (18.2%) permitted animal viability but altered the GMR>dCsk-IR phenotype. Of these, 251 drugs had an enhancing effect, and 99 compounds (5.0%) had a suppressive effect. 6 drugs had different effects (suppression vs. enhancement) at different concentrations. The 99 compounds that initially suppressed the GMR>dCsk-IR phenotype were re-tested in multiple wells. 39 compounds demonstrated consistent phenotypic suppression
Reducing Hh activity (top) suppressed the MMP activity reporter DQ-Gelatin and (bottom) expanded Hh pathway activity.

Table 39 NCI Diversity Set compounds suppressed the dCsk-IR phenotype.

(See below)
through chemical inhibition of Hh signaling suppressed MMP activity (Figure; Vidal et al, submitted). Further, reducing Hh pathway activity in the eye mildly but consistently suppressed the GMR-dCsk-IR phenotype, while over-expression of the Hh pathway effector Ci enhanced (not shown). These genetic data provide further support for the model that Hedgehog signaling plays an important role in Csk/Src-mediated metastasis. I am not proposing to follow through on the role of Drosophila Hedgehog because this is an excellent beginning project for Marcos Vidal as he starts his own laboratory.

**Mouse validation:** The Weilbaecher laboratory then demonstrated that Jervine and Cyclopamine suppressed expression of the Hedgehog target Gli-1 in both 4T1 breast cancer cells and B16 melanoma cells (not shown) and reduced proliferation of fluorescently-labeled 4T1 breast cancer cells in a cell culture dish (Figure). A similar reduction in proliferation was observed in 5/6 other compounds we tested that were identified from our fly screen. In a xenograft model, daily injection of 1 or 50 mg/kg of Jervine or Cyclopamine consistently prevented metastasis of 4T1-luc-GFP cells (10^5 cells injected into the left ventricle of Balb-C mice) to the lung (but not bone); by day 9, tumor mass was reduced an average of more than four-fold (Figure). This data provides important validation that our fly models are able to identify candidate therapeutic compounds that show efficacy in a standard mouse xenograft model.

**KEY RESEARCH ACCOMPLISHMENTS:**

1) Identification of 6 compounds that decrease metastasis in Drosophila metastasis model and decrease viability of mammalian breast cancer cells in vitro.
2) Validation of the compound Cyclopamine, a hedgehog inhibitor, to block lung metastases in murine breast cancer xenograft.
3) This work provides a template for moving candidate therapeutic compounds from fly to mouse.
4) The Drosophila and mouse data demonstrate an important connection between Src and Hedgehog signaling in mediating metastasis.

**REPORTABLE OUTCOMES:**


**CONCLUSIONS:**
We propose to combine our expertise to target a process that is critical to metastasis that is likely conserved in flies, mice and humans. The advantages of addressing the question of metastasis through the combined expertise of the Cagan and Weilbaecher labs is that we will use the powerful genetic tools provided by Drosophila that will identify key genetic pathways critical to tumor cell migration and metastasis that can be rapidly and rigorously tested. This a real time, in vivo dynamic screen that occurs in a whole organism. Tumors develop in the epithelial layer of the wing and the genetics of tumor cell invasion and migration throughout the organism can be modeled in real time, and genetically manipulated in large scale genetic screens. Dr. Weilbaecher’s laboratory will take advantage of the genetic knowledge gained from the Drosophila metastasis models in the development of an improved breast cancer metastasis mouse model. Dr. Cagan’s laboratory will be provided with mammalian human and murine breast cancers to validate their genetic and pharmacologic anti-metastasis strategies. Jointly, Drs. Cagan and Weilbaecher propose to develop novel therapeutics targeted to the metastatic process. In year one, we have identified 6 compounds that decrease metastasis in Drosophila metastasis model and decrease viability of mammalian breast cancer cells in vitro. We have validated the compound Cyclopamine, a hedgehog inhibitor, to block lung metastases in murine breast cancer xenograft and will use this as a template for testing other candidate therapeutic compounds from fly to mouse. Finally, we have uncovered a previously unknown and important connection between Src and Hedgehog signaling in mediating metastasis.

REFERENCES:
None

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None