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Progress Report for Moran Foundation 2005: Identification of a novel signaling pathway crucial for blocking hormone-dependent malignancies

Scott Goode, Ph.D., Department of Pathology

(1) Progress Report:

The proposal had two aims:

(i) Determine how Warts and DAIB1 Cooperate with Dlg. *wts* is a tumor suppressor that encodes a Ser/Thr kinase of the cAMP-dependent kinase superfamily. Wts is in a complex with two additional tumor suppressor proteins, Salv and Hpo. We will determine the precise role of Wts/Salv/Hpo in regulating FC polarity, proliferation, and/or motility. We will determine if Wts/Salv/Hpo is downstream of Dlg, and how it transduces signals to DAIB1. Since nothing is known about the upstream signals regulating Wts, or the downstream mediators of Dlg, this Aim will potentially bridge two intensively studied tumor suppressor pathways highly relevant to mammalian and human tumor biology.

(ii) Determine how Ebi and DAIB1 Cooperate with Dlg. *ebi* encodes for a F-box and WD40-repeat protein that promotes protein degradation of specific targets, including junction molecules and SMRTER/SMRT, a corepressor required for AIB1 activation. We will determine what proteins Ebi targets in follicle cells to suppress invasive tumorigenesis, and the function of Ebi in polarity, proliferation, and invasion. We will determine if Wts acts with Ebi to regulate SMRTER, or if Wts acts parallel to Ebi to regulate DAIB1 directly. This Aim will establish how Dlg regulates AIB1 via Ebi and SMRTER.

For Aim 1 we have established that Wts and Mats act downstream of Fas2 and Dlg to suppress invasion, but not Salv and Hpo. The evidence is as follows. First, loss of one copy of *wts* or *mats*, but not Salv or Hpo enhances *dlg* or *Fas2* invasion. Second, complete loss of *wts* or *mats* causes loss of DAIB1, similar to *dlg* or *Fas2*. Third, over expression of Wts or Mats, but not Hpo or Salv, suppresses *dlg* and *Fas2* invasion. To further demonstrate that Wts and Mats act downstream of Fas2 and Dlg, we are complementing this *in vivo* genetic evidence with *in vitro* cell culture experiments, aimed at testing the dependence of DAIB1 transcriptional activity on Fas2-Dlg and Wts-Mats.

For Aim2, the available evidence indicates that Ebi acts independent of the Fas2-Dlg-Wts-Mats-DAIB1 pathway. We found that *ebi* and *wts* and *mats* all enhance *Fas2* and *dlg* invasion, and cause DAIB1 loss. However, whereas *ebi* causes loss of SMRTR, *wts* and *mats* cause SMRTR to be up regulated. Since all three mutants function with *Fas2* and *dlg* to suppress invasion, this suggests that Ebi acts independent of Wts and Mats to regulate DAIB1, through a parallel pathway that directly regulates the SMRTR

complex, and thus indirectly influences DAIB1 levels through corepressor. Additional evidence that *Ebi* acts in a parallel pathway is that although it enhances *dlg* invasion, unlike other enhancers, it does not causes invasion tumors on its own. Based on these results we do not plan to pursue work on Ebi further.

(2) Presentations that acknowledge Moran Support:

All presented at the 47th Annual *Drosophila* Research Conference, 2006.

Goode, S., Zhao, M., Hall, C. and Szafranski, P. Baylor College of Medicine, Department of Pathology.

A Basolateral Junction Signaling Pathway Suppresses Normal and Tumor Invasion (Talk)

Our lab uses the fly ovary to dissect molecular mechanisms underpinning normal and tumor cell invasion. We have screened mutations in components of all of the epithelial junctions in the follicular epithelium, and find that only components of the basolateral junction lead to early cell invasion and suppress normal cell migration. In contrast, mutations in components of the apicolateral, adherens, and basal junctions do not lead to invasion. Further, we find that only components of the basolateral junction change expression at the time of normal cell motility, indicating the specificity of the basolateral junction in suppressing invasion. To identify a basolateral pathway that regulates motility, we completed a genomic screen for molecules that enhance discslarge and fas2 invasion. We identified a pathway by which basolateral junctions signal through Wts and Mats kinases to regulate expression of hormone-dependent nuclear transcription through Taiman, the fly ortholog of Amplified in Breast Cancer-1. AIB1 appears to feedback to regulate localization of other epithelial junction molecules crucial for motility. Others and we have found that several molecules in this pathway are misregulated in human ovarian and other hormone-dependent cancers. However, our data brings a functional order to these molecules as components of a previously unknown basolateral signaling pathway. The pathway may serve as an integration point between hormonal signals and regulation of growth and motility of normal and cancerous cells.

Hall, C., Zhao, M., Szafranski, P., and Goode, S.

Connecting Tumor Suppressors: Ebi and Wts in Dlg Signaling (Poster)

Approximately 90% of human cancers arise from epithelial tissue and a pivotal event in cancer progression is the ability of tumor cells to invade neighboring tissues and metastasize. Our lab uses the *Drosophila* follicular epithelium to model the cell polarity, migration, and invasion aspects of human cancers. Loss of Discslarge (Dlg), the *Drosophila* homolog of human ZO-1 and hDLG, in follicle cells causes them to overproliferate, lose polarity, and invade between germ cells, much like human cancer.

Dlg is scaffolding protein that localize to sites of cell-cell contact where it binds and clusters adhesion, signaling, and cytoskeletal molecules. Since little is known about the signaling partners that cooperate with Dlg to regulate epithelial polarity, proliferation, and migration, our lab performed a genetic modifier screen to identify components of the Dlg pathway. Among others, Ebi and Warts were identified as Dlg enhancers. Ebi encodes a WD40 repeat protein involved in cell proliferation control and hormone dependent transcriptional activation. Warts encodes a Ser/Thr kinase that cooperates with three tumor suppressors, Hpo, Sav, and Mats, and a potential oncogene, Yki, to suppress tumorigenesis. To better understand the function of Ebi, Warts, and their partners in Dlg signaling, we are characterizing their role in cell proliferation, polarity, and migration in the Drosophila follicular epithelium. We see that both Wts and Mats, but not Hpo and Sav, enhance Dlg tumorigenesis and are required for proper follicle cell polarity and proliferation control. In addition, all of these molecules, as well as Dlg, modulate the levels of DAIB1, the *Drosophila* homolog of Amplified in Breast Cancer-1, a steroid receptor coactivator. Furthermore, we see that, like DAIB1, some Wts signaling molecules regulate the hormone dependant process of border cell migration suggesting a previously unknown role for the Warts tumor suppressor pathway in cell migration. We have developed a hypothetical model in which Dlg signals to Ebi and Wts to regulate hormone dependant transcription through DAIB1.

Zhao, M., Hall, C., Szafranski, P. and Goode, S.

Basolateral junctions signal through Wts/Mats tumor suppressors to regulate hormone-dependent transcription (Poster)

Abstract

We are using *Drosophila* egg chamber as an animal model to study epithelial motility. During oogenesis, border cells delaminate from the follicular epithelium and migrate to the oocyte. Basolateral junction protein Fas2 suppresses motility, yet is required for border cell delamination. To determine how Fas2 controls delamination, we screened four transcription factors that are required for border cell motility, to determine if Fas2 is required for their expression. Only reduction of taiman (known as DAIB1, the *Drosophila* homolog of human Amplified in Breast cancer-1) is lost in *Fas2* mutants, the other three transcription factors showed no change. To further investigate how Fas2 targets DAIB1, we screened for enhancers of Discslarge (Dlg), a Fas2 binding partner. We identified Lethal giant larvae (Lgl), Scribble (Scrib), Warts (Wts), Mats, and Ebi as interactors that enhance *dlg*. DAIB1 is mislocalized in *dlg*, *wts, mats*, and *ebi* clones, but not *lgl* and *scrib*. We have thus identified a novel basolateral signaling pathway in which Fas2/Dlg targets Ebi-DAIB1 through Wts and Mats tumor suppressor kinase.

(3) The aims of the project outlined in the proposal have been completed. The project is on going as indicated in the progress report.