RE: Summary Report for Moran Foundation

Identification of Polypeptides that Regulate Epithelial-Mesenchyme Transition

Principal Investigator: Scott Goode, Ph.D.

Specific Aims

To deepen our understanding of the molecular mechanisms underlying Dlg invasion- suppressing activity we are conducting the following experiments:

1. Testing Dlg domains for EMT . We will construct transgenic animals in which individual Dlg domains (PDZ1, PDZ2, PDZ3, SH3, membrane 4.1 binding site, guanylate kinase) have been deleted, either singly, or in combination. We will determine the degree to which these mutant molecules block cell invasion during oogenesis. These experiments will define Dlg domains and/or combinations of domains specifically required for suppressing cell invasion.

2. Isolation of partial cDNAs to proteins that interact with Dlg EMT domains. We will conduct interaction trap screens to identify ovarian proteins that bind to Dlg invasion-suppressing domains. We will obtain full-length cDNAs for these proteins, determine their primary structure, confirm that they expressed in ovarian tissue by *in situ* hybridization, and use biochemical techniques to confirm that the proteins interact with the appropriate Dlg domain.

These experiments will reveal the structure of proteins that directly interact with Dlg invasion suppressing domains, and help us to generate hypotheses about how they cooperate with Dlg to block cell invasion. These hypotheses will be tested in future genetic and cell biological experiments that take into account the unique structural characteristics of each molecule.

Progress to Date

Aim 1

We have constructed all 14 of the proposed P-elements for making transgenic *Drosophila* in which individual Dlg domains have been deleted, either singly, or in combination. We have transformed *Drosophila* with these P-elements. We have established multiple homozygous independent lines for each construct. We have constructed a special X-chromosome that allows us to simultaneously remove endogenous Dlg while turning on transgenic Dlg. We are crossing the transgenic lines into the special X-chromosome background so that we can determine the degree to which these mutant molecules block cell invasion.

Aim 2

We have used the interaction trap system to show that a candidate PDZ-binding protein called Kek1, binds to Dlg PDZ1 and PDZ2. We have shown in control experiments that binding depends on a critical motif at the carboxyl-terminus of the Kek1 protein that is expected to interact with the PDZ domains. Interestingly, the gene encoding Kek1 is expressed at the poles of the egg chamber starting from the earliest stages of oogenesis, in a tissue pattern that resembles the pattern of cellular over-proliferation in Dlg mutant egg chambers. Further, we have shown that Kek1 mutations genetically interact with Dlg mutations, and with mutations in a second Dlg PDZ-binding protein called FasII. Thus, by several criteria, Kek1 appears to be an authentic Dlg PDZ-binding protein. We are generating

sera to Kek1 so that we can determine the detailed localization of the protein in ovarian tissue, and so that we can confirm that Kek1 binds to Dlg using immunoprecipitation experiments. We are also in the middle of working out a new technique for assaying cell migration *in vivo* so that we can determine the impact of Kek1 and FasII on cell migration.

List of Publications Resulting from this Support

This work is not ready for publication. We are presently working on two manuscripts and are planning experiments for two manuscripts following publication of the first two this year.