Moran Foundation: Progress Report

Title: Identification of Proteins Interacting with the Cytoplasmic Domain of Cadherin 11, a New Type II "Mesenchymal Cadherin" (97-0092)

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Progress Report: The goal of this project is to identify intracellular proteins that interact with the cytoplasmic domain of cadherin 11, a type II cadherin that we found to be abundantly expressed in developing vascular smooth muscle. Two approaches were planned: (1) Use yeast two-hybrid interaction cloning methods to screen for binding partners in a 12.5 day mouse embryo cDNA library, and (2) Immunoprecipitate full length vs truncation mutants of cadherin 11 in "pull-down" assays to directly assess binding partners by immunoblotting analysis. The first approach does not require prior knowledge of the binding partner and we hoped to identify new interaction partners by this method. The second approach is a "candidate factor" approach which tests for interactions of cadherin 11 with known proteins.

We first used a commercial Gal4 yeast two-hybrid system but found that the complete cytoplasmic domain (CD) of cadherin 11 was strongly self-activating in this system. We then tested a construct containing the proximal half only of the cadherin 11 cytoplasmic domain (PD), but that too was strongly self-activating. We then switched to a LexA two-hybrid system in yeast L40 cells that we obtained from Dr. Pierre McCrea, MD Anderson, that utilized a VP16 activation domain construct. While the full length cadherin 11 cytoplasmic domain construct also strongly self-activated in the LexA system, the proximal domain construct did not. A prey library (VP16-fused ED10.5 mouse embryo) was screened (1.5 million clones) with the proximal domain construct as bait and 342 His3-positive colonies were picked for restreaking. Twenty-seven of the 342 original colonies showed robust growth and were also lac-Z positive in a filter lift assay. Upon further testing all 27 independent colonies obtained after the second screen were found to be false-positives. A new bait vector was prepared containing the proximal domain plus an additional 30 amino acids of the intracellular domain, however this too strongly self-activated in the LexA system.

The "candidate factor" approach using cadherin 11 pull down assays has been more successful. Using MC3T3-1 cells, from which cadherin 11 was originally cloned, we have shown that two cytoplasmic proteins (β-catenin and p120) naturally interact with cadherin 11 in these cells. Upon stimulation with PDGF, a new protein of 70kDa becomes tyrosine-phosphorylated and associates with the cadherin 11 complex. We have identified two domains of cadherin 11 localization in MC3T3-1 cells, namely at cell-cell borders and focal adhesions. We are continuing to examine the interactions of cadherin 11 with intracellular binding partners in MC3T3-1 cells to better understand how the cell-cell adhesive and signaling functions of cadherin 11 are regulated in mesenchymal cells.

Presentations that Included Some or All of This Work:

(1) University of Virginia, Department of Physiology and Biophysics, October, 1997.
(2) University of North Carolina, Chapel Hill, Departments of Pathology and Molecular Genetics, February, 1998.
(3) Biogen, Inc., Cambridge, MA, April, 1998.
(4) Gordon Conference on Vascular Cell Biology, June, 1998.

Publications that Included Some or All of this Work:

Full Length Paper

(1) "Molecular Cloning of Chick Cadherin 11 and its Expression During Formation of the Vascular Tunica Media", J. Wei, XR Dong, S. Topouzis, W. Zimmer, F. Broders, JP Thiery, V. Koteliansky, MW Majesky. *Circ. Res.,* in revision.

Abstracts

(1) "Origins of Vascular Smooth Muscle Diversity", MW Majesky, S. Topouzis, J. Wei, XR Dong, T. Landerholm. J. Mol. Cell. Cardiol. 29:A267, 1997.
(2) "Molecular Cloning and Expression of Cadherin 11 During Development of the Aortic Tunica Media", J. Wei, XR Dong, S Topouzis, T. Landerholm, V. Koteliansky, F. Broders, JP Thiery, MW Majesky. *Circulation* 94:I-119, 1996.