



2006 Progress Report
Caring for Carcinoid Foundation Research Grant
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“Proteomic analysis of GI neuroendocrine tumors”

The overall goal of this project is to determine whether there are unique protein expression patterns in gastrointestinal (GI) neuroendocrine tumors that can provide novel insights into disease pathogenesis. Through this approach, we hope to identify specific therapeutic targets that have been previously unrecognized. This strategy has never been undertaken in the study of GI neuroendocrine tumors and is a powerful, unbiased way to identify some of the key proteins involved in tumor development.

Our strategy was to examine human tumor samples, in contrast to cultured cell lines. While there is some inherent tissue heterogeneity, this approach is appealing in that the proteins identified are likely to be functionally relevant in actual tumors. We began our analysis with pilot experiments to optimize protein solubilization techniques with samples of normal human pancreas. Gel electrophoretic studies revealed a clean separation of total protein. The gel was then divided into 22 fractions based upon protein size, and proteins from each fraction were analyzed by mass spectrometry. A total of 997 distinct proteins were definitively identified, many of which are known to be present at very low concentrations. We were then able to proceed with analyses of tumor samples, and we chose to compare neuroendocrine tumors of different stages in an effort to identify proteins that are important in tumor progression (i.e. what makes a benign tumor turn into a malignant tumor). In the total proteome, 1160 unique proteins were identified in the benign group, and 1684 proteins in the malignant group. 770 of these proteins overlapped both groups. Our focus was on the proteins expressed in the malignant group but not in the benign group.

In addition to total protein analyses, we have completed sub-fraction analyses, looking at phosphorylation status through 2 independent techniques (phospho-peptide fragment and whole phospho-protein analyses). Protein phosphorylation is a key mechanism regulating the activity of many cellular processes and phospho-status can be assessed with current proteomic technologies. In these analyses, 150 unique phosphoproteins were identified in the benign group, and 261 in the malignant group. 91 of these were present in both groups. The data sets were then analyzed through a variety of software programs (Ingenuity, David Bioinformatics) to determine whether the proteins identified in the malignant group clustered within specific signaling pathways. Through this analysis, several candidate genes emerged. We have chosen to analyze two of these, 14-3-3 and CA8.

Validation of these candidates required analysis of human tumor tissues, and for this purpose we constructed tissue microarrays (TMAs) through a collaboration with Dr. Vikram Deshpande in the MGH Pathology department. 65 neuroendocrine tumors were assembled onto two TMA slides. Preliminary studies have been completed utilizing antibodies for these 2 proteins. Final conclusions will be made after clinical characteristics of all the cases has been completed. In this manner, we will be able to make meaningful correlations between the protein levels of these genes and clinical features, such as stage of disease.

After validation of these as well as other candidates, the next step in this process will be to perform functional assays in cultured cells to determine how these proteins are controlling the tumorigenic process. With this mechanistic information, we hope to gain new insights into the molecular pathways that underlie the development of these poorly understood tumors and thereby position ourselves to translate these findings into meaningful applications of therapy and prognosis.