

Proteomics of Prostate and Ovarian Tumor Cells Using Multidimensional Liquid Separations, Mass Mapping and Protein Microarrays

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The protein expression of prostate and ovarian tumor cells are studied to search for potential markers of cancer. Fresh frozen tumor cells are obtained and prepared for study by the pathology groups at the University of Michigan Medical Center. The tumors are lysed and separated using a two-dimensional liquid separation, which is capable of separating out nearly 3000 protein bands. This method involves separation based upon pI in the first dimension using chromatofocusing and by hydrophobicity in the second dimension using nonporous silica(NPS) RP-HPLC. The liquid eluent from the second dimension is then split and 1/3 is directed to an electrospray time-of-flight mass spectrometer (ESI-TOF MS) for mass analysis, while the remaining 2/3 is collected by a fraction collector for MALDI-TOF MS analysis. The result is a mass map as a function of pI analogous to 2-D gel electrophoresis. The ESI-TOF MS provides an accurate MW value, which can be used with MALDI-TOF MS peptide mapping and MS/MS for accurate identification of proteins in these human cancer cells. In addition, the MW value provides a means to determine whether a protein is modified including the presence of truncations, deletions, sequence changes and splice variants. Using this method large numbers of tumor samples can be mapped and each type of cancer can be classified according to its protein profile. In addition, liquid protein microarrays can be used to map global changes in phosphorylations and glycosylations. Using PCA and hierarchical clustering methods marker proteins have been identified which can be associated with different grades and stages of cancer progression and different subtypes of the disease. The method provides a means for pathologists to identify the specific type of tumor, i.e. whether it is more benign or malignant, and consequently determine a specific treatment for that disease.