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Title: Discovery and Identification of Interacting Proteins of the Growth Factor Receptor Bound Protein (Grb2) Utilizing SELDI ProteinChip® Technology

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Novel Aspect: Discovery of novel interacting proteins of Grb2 using affinity chemistry with chromatographic fractionation, detection by TOF-MS, identification by MS/MS.

Key Words: Affinity, Chromatography;

Introduction:

One of the major challenges that researchers currently face involves simplifying and understanding the complexity of protein-protein interactions essential to numerous cellular functions. Using SELDI (Surface-Enhanced Laser Desorption/Ionization) -TOF MS based Interaction Discovery Mapping™ (IDM) platform, we are able to directly detect multiple interacting proteins simultaneously. This technology platform captures, enriches and detects multiple interactors in a single analysis. The Grb2 protein is required during embryogenesis for the differentiation of endodermal cells. By determining the key interacting proteins of Grb2, we may gain a better understanding of how Grb2 regulates embryogenesis.

Methods and Instrumentation:

Grb2 was recombinantly expressed as a GST-fusion tag (supplied by Prolexys, Salt Lake City, UT) and immobilized onto carbonyldiimidazole-activated ProteinChip arrays (RS100) or affinity beads for increased sensitivity. Grb2 interacting proteins were then captured from protein extracts of human kidney embryonal (HEK) cells (supplied by Prolexys). Interacting proteins captured on Grb2-coupled RS100 arrays were directly detected and analyzed on the PBSIIC TOF-MS. Interacting proteins captured on Grb2-coupled beads were eluted and detected on Q10, nickel coated IMAC30, and CM10 ProteinChip arrays. Interacting proteins were further enriched and identified by protease digestion and analysis on the ProteinChip Tandem MS (QSTAR) Interface.

Preliminary Data:

Utilizing this protein discovery platform, we were able to capture and identify Grb2 and several interacting proteins from HEK cell extracts. While specific capture of Grb2 was observed on both the RS100 arrays and the affinity beads, protein capture on Grb2-specific affinity beads followed by detection on chromatographic arrays allowed for increased binding capacity and sensitivity, thereby increasing the total number of Grb2 interacting proteins detected. Mass spectra corresponding to eluted proteins profiled on the CM10 arrays showed a higher number of Grb2 specific peaks when compared to spectra from the IMAC-Ni and Q10 arrays. Several protein peaks were selected for subsequent purification and identification. Following Grb2 specific capture, interacting proteins were purified on a CM10 resin and subjected to protease digestion. Resultant peptides were applied to SEND™-ID arrays for rapid MS/MS identification. SEND arrays are a matrix-free mass spectrometry surface yielding increased sensitivity of peptide detection and lower background noise. The Grb2 interacting proteins identified included SOS-1, Dynamin-2, HSP90 and HSP70 which have been reported previously in the literature as well as several novel proteins. The use of SELDI-TOF MS based protein discovery mapping resulted in the rapid discovery, purification, and identification of Grb2 specific interacting proteins.