Phospho-PepArray™ based Identification of Novel Protein Interaction Networks in Tyrosine Kinase Signaling Pathways

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Introduction

Protein phosphorylation mediates many cellular responses and is essential for many biological functions during development. About one-third of all cellular proteins are phosphorylated, representing the phosphorylated proteins, and phosphorylation can alter a protein’s function, activity, localization and interaction.

Tyrosine phosphorylation events mediated by aberrant activation of Receptor Tyrosine Kinases (RTK) pathways have been proven to be involved in the development of several diseases including cancer.

With the available phosphorylation data on various high-throughput proteomic technologies, it is becoming increasingly evident that many proteins are phosphorylated in multiple, multiplex signaling pathways through multiple protein-protein phosphorylation sites (T/P and Y/P). Hence it is becoming extremely hard to target a specific protein as a biomarker or for a drug.

Post-translational modifications (e.g., PTMs) on proteins create multiple subgroups of the same proteins which are pathway specific. Hence a scientific strategy is required to cluster these phosphorylated sites that are pathway specific.

Here we present our microchip based PepArray™ peptide microarray technology to characterize and identify novel protein interactions from cancer signaling pathways.

The interaction between phosphopeptides (PPEPs) and phosphoprotein binding domains containing protein (PPBDs) on a microchip reveal novel protein cascades representing various signaling pathways through target binding.

More than 165 families of PPBDs (SH2, PTB, WW, WW-PPP) represent a vast majority of protein in a signaling cascade. Peptides with phosphotyrosine modifications not only bind to proteins with SH2 domains but also activate downstream proteins (kinases and phosphatases) to initiate a signaling cascade.

We characterize PhosphoRTK (PTK) peptide interactions on an SH2 domain containing protein. GRB2 is identified protein complexes under various signaling cascades. We have used Breast cancer cells (T47D) and Human breast cancer cells (MCF-7) to screen peptides (PepArray®) to demonstrate the differential signaling cascade mediated by differential protein-protein interaction networks.

Further focus will be on technology advancement through integration of high-throughput peptide microarray with computational tools to rapidly identify novel protein interaction networks in various cancers and non-oncogenic diseases and increase the sensitivity of detecting protein interactions.

The human and mouse complement of SH2 domains are known substrates of SH2 domain containing proteins (1). A SH2 domain interacting phosphopeptide (SH2-PPEP) microarray was designed. Unique PPEP: PPEP that uniquely binds to a SH2 domain. Overlapping among the four SH2 proteins.

SH2-2-PPEP Microarray Design and Analysis

Statistics of SH2 proteins and PPEPs in the microarray

- SH2 proteins
- PPEPs
- PPEPs interactions
- SH2-2-PPEP

The Microarray is used for identifying different binders of Grb2 in two cell lines.

SH2 Protein Network and Cell Surface Receptor Pathways

Detection of PPEP mediated protein Binding

Recombinant SH2 protein and cell lysate binding assays

Recombinant SH2 protein binding assay

SH2 proteins

Protein carbon drawings with purple ellipse are SH2 proteins

Methods and Tools

PepArray pro – a user-driven PepArray design program

Signatures Peptides/Panels of Four SH2 Proteins

Direct and Indirect Grb2 mediated binding of PPEPs

Grb2 in Cell lysate binding assay

Summary

A strategy has been outlined for identifying signature peptide probes that could serve as signaling pathway specific markers to distinguish normal and diseased protein samples (e.g., Identification of cancer types and subtypes).

By combining in silico analysis (PepArray pro) and binding experiments, we screened signature peptides/panels for Grb2. BTK, Src and ZAP70. With signature peptides of Grb2, we could detect Grb2 in cell lysates with sensitivity of low nM level.

We demonstrate a method to investigate SH2 protein interactions and related pathways in cells. From in silico analysis and recombinant protein binding assay to cell lysate binding assay, we found differential binding patterns of GRB2 protein in cell lysates (direct binding) which lead us to identify Grb2 mediated SH2 protein networks and related pathways (ppTyr and CD74) cell.

The future direction is to develop this technology for Biomarker screening in patient sample, focusing on personalized medicine to help physicians to diagnose, select and treat treatment options for diseases.

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References