Microarray-Based Phospho-Proteomic Profiling of Complex Biological Systems

C. Rory Goodwin MD, PhD; Crystal Woodard; Xin Zhou; Alessandro Olivi MD; Jonathan Pevsner; Heng Zhu; John Laterra MD, PhD

Department of Neurosurgery, Pharmacology and Molecular Sciences, and Neurology, The Johns Hopkins University School of Medicine

Hugo W. Moser Research Institute at Kennedy Krieger, Baltimore, MD, USA.

Learning Objectives

By the end of this session, participants should be able to discuss how microarray-based technologies can be used to assessing intracellular signaling events applicable to human oncogenesis.

Introduction

Protein phosphorylation influences ~30% of the proteome and regulates fundamental cellular processes such as cell proliferation, migration, and angiogenesis. Protein microarray technology has been successfully used for identifying substrates of purified activated kinases. In this study, we describe the novel application of protein microarray technology to globally assess the influence of PTEN function on the phospho-kinome of glioma cells in vitro and glioma xenografts in vivo.

Methods

We utilized a glioma cell line engineered to express PTEN via a tet-on promoter system (U87-tetPTEN) and validated results in clinical pathological specimens. Whole cell (in vitro) or whole tumor (in vivo) lysates obtained from control and doxycycline-treated conditions were incubated on human protein microarrays in the presence of 32P-?-ATP and kinases that demonstrated a significant reduction in phosphorylation in the PTEN induced group (doxycycline-treated) were identified.

Results

- PTEN was robustly increased, Akt phosphorylation was inhibited by ~90% after incubating cells with doxycycline in vitro for 24 hours.
- PTEN reconstitution inhibited cell growth in vitro and treating animals following cell implantation with doxycycline arrested the growth of subcutaneous tumor xenografts. Using the protein microarray, PTEN reconstitution decreased the phosphorylation of 135 protein targets in vitro and 103 protein targets in vivo.
- Twenty clinical WHO grade IV gliomas were obtained and stratified by semi-quantitative immunoblot analysis into high phospho-Akt expressers and low phospho-Akt expressers. Tumor lysates were analyzed by immunoblot for five phospho-proteins shown to be PTEN-regulated by the protein microarray assays.
- As predicted by microarray analysis, in comparison to the high phospho-Akt glioblastomas, the low phospho-Akt glioblastomas had statistically significantly lower phosphorylated forms of the kinases.

Conclusions

Thus, microarray analysis could be potentially used to predict associations between Akt activity and protein phosphorylation in clinical specimens. Our findings identify a novel microarray-based method for assessing intracellular signaling events applicable to human oncogenesis.

References

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