Phosphoproteome of PTEN-Null and PTEN-Expressing U-87 MG Human Glioblastoma Cells using a Phosphochip – QTOF MS platform

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Introduction

Glioblastoma (GBM) is the most common primary brain tumor. Genetic alterations in PTEN a lipid and protein phosphatase and negative regulator of PI3K pathway is found in 60% of GBMs which correlates with patient outcome and hence is an attractive therapeutic target. Global impact of PTEN status, and so PI3K- AKT pathway activity, which involves many serine/threonine-kinases, has not been studied in GBM. Recent reports also suggest that PTEN may affect the stability of specific proteins by targeting them for degradation. We compared total proteome and phosphoproteome of glioma cells having different PTEN status to understand differential phosphorylation and expression of proteins. This information will further help us study effect of some candidate PI3K inhibitors in gliomas

Materials and Methods

We used PTEN null U87MG glioma cell line and an engineered U87MG cell line where wild type PTEN has been re-expressed. These two different cells were grown in standard tissue culture conditions and protein extraction done in urea buffer. After trypsin digestion peptides were separated by strong cation exchange (SCX) chromatography using ammonium formate gradient from 5mM pH2.8 to 500mM 6.4. Thirteen SCX fractions were collected and injected onto a Microfluidic RP-TiO2- RP HPLC Phosphochip and sequential analysis of nonphospho as well as phosphopeptides were done using 6520 Q-TOF system (Agilent Technologies). MS data thus generated was searched against Human IPI database using Spectrum Mill proteomics software

Table 1. Total Number of Distinct Proteins Identified in Glioblastoma Cells

	Total proteins	Total Peptides	Total Phospho proteins	Total phospho peptides
Total	2509	15002	373	1182
U87_MG PTEN null	2216	11593	316	945
U87_MG PTEN	2081	10840	237	630

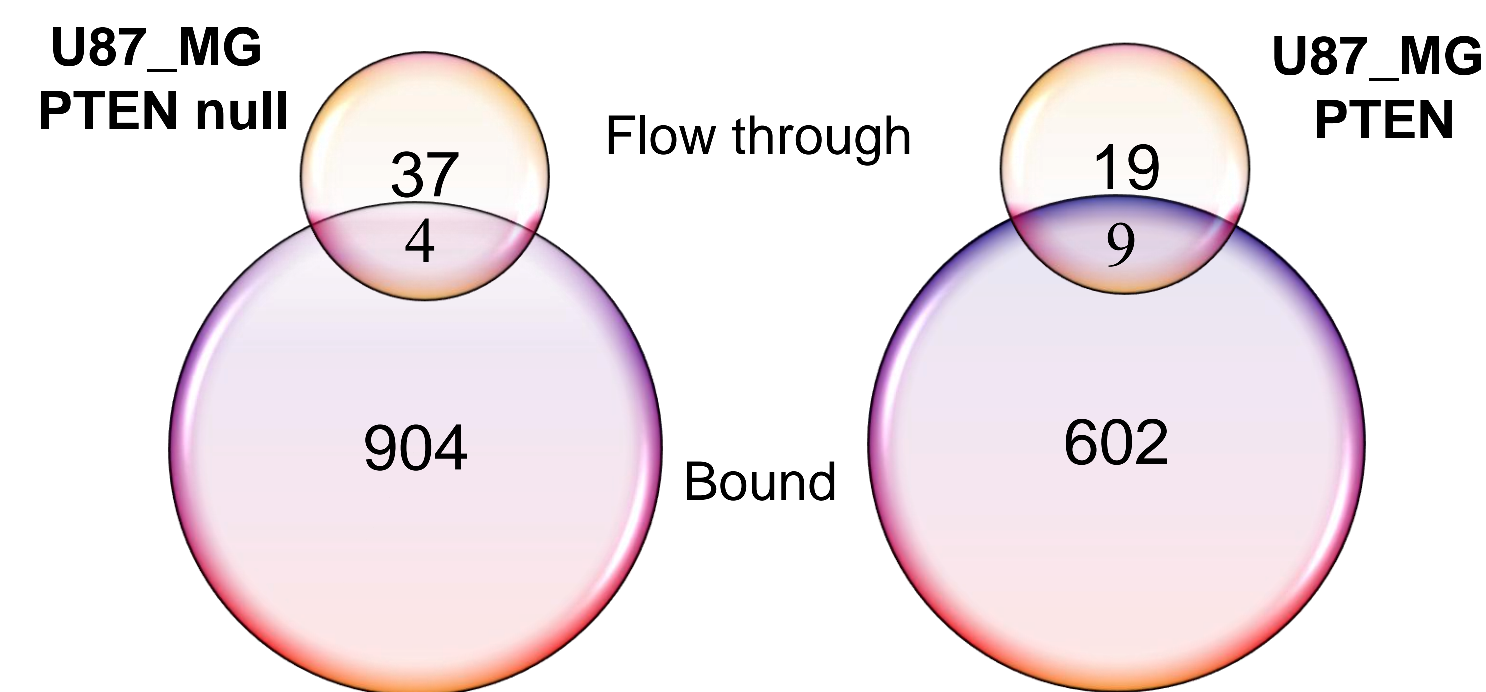

Fig.1 : Distribution of phosphopeptides on Phosphochip

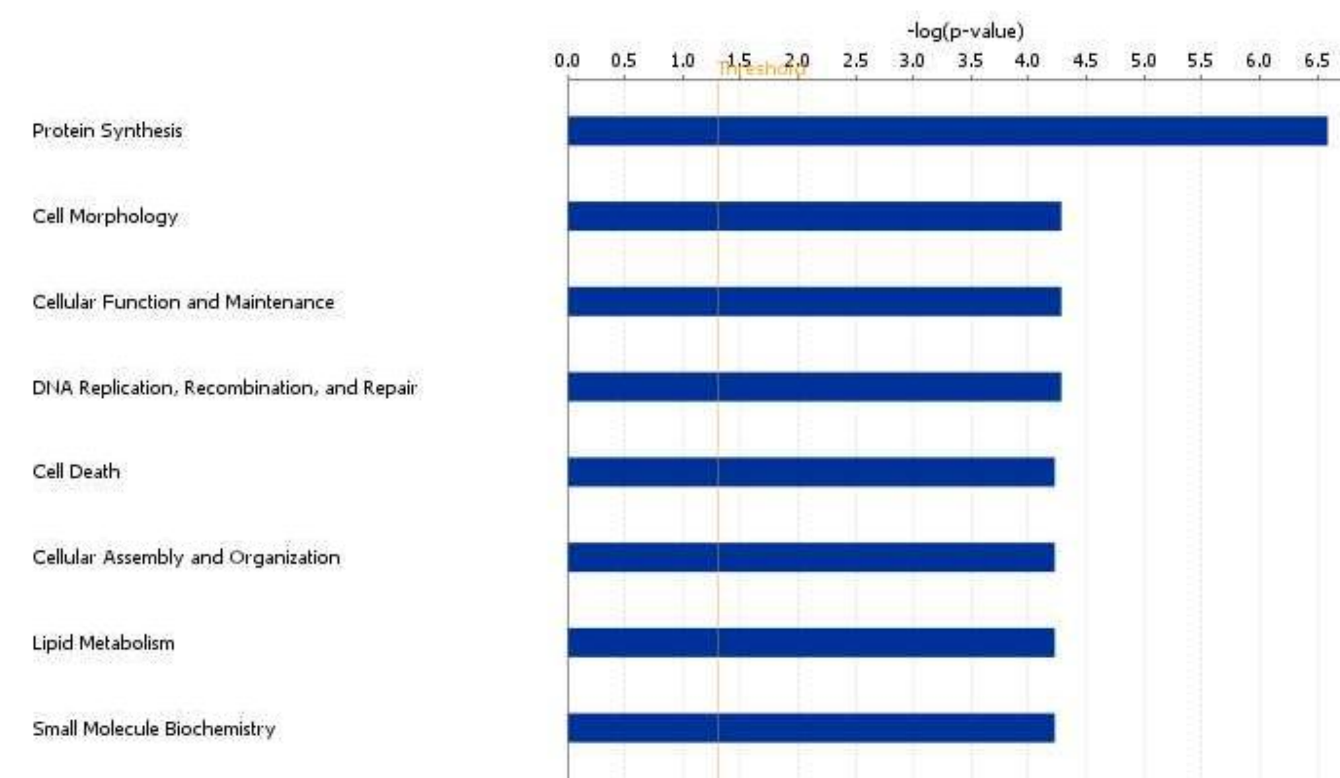

Fig.2 : Functional Analysis of overexpressed proteins in U87MG-PTEN cells

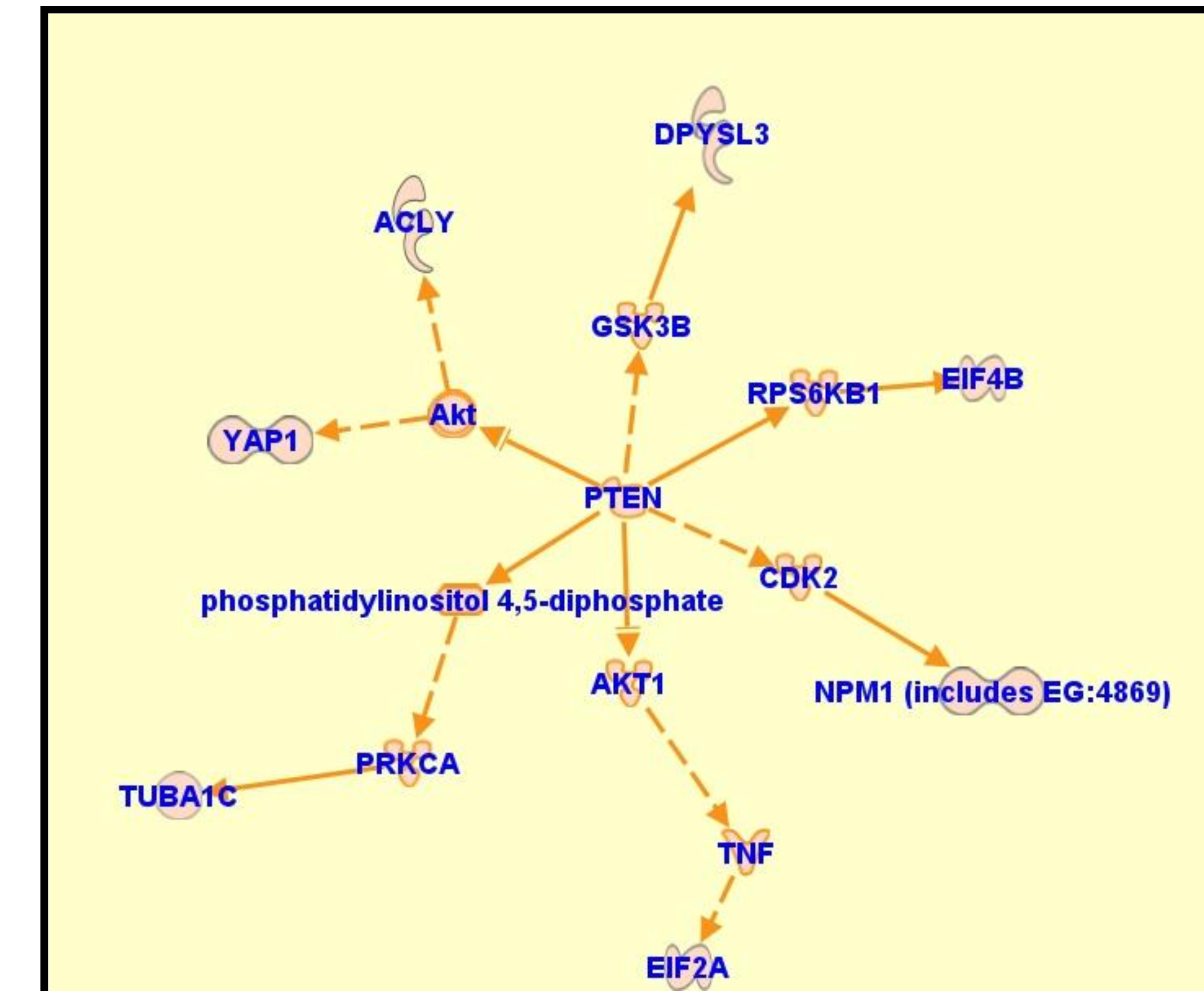

Fig.3: Ingenuity pathway analysis of select phosphoproteins showing decreased phosphorylation that are known to be downstream of PTEN

Results

1. We identified total of 2509 unique proteins (0.15% FDR) and out of this 373 are unique phosphoproteins (Table 1)
2. Almost all phosphopeptides (>96%) were bound to TiO2 (Fig.1)
3. Of the total proteome, 140 proteins showed higher expression in PTEN expressing cells while 60 proteins showed lower expression. Overexpressed proteins are involved in important cellular functions, (Fig. 2)
4. U87 PTEN null cell lines have significantly more number of phosphoproteins (Table 1)
5. Of the proteins showing significant decrease in phosphorylation quite a few are known to be downstream of PTEN (Fig.3)

Summary

- Use of Phosphochip in combination with separation technique such as SCX is an effective tool to study total proteome and phosphoproteome together.
- We could identify known downstream targets of PTEN phosphatase, and extending this to other targets will help in identifying kinases affected by PTEN.
- There are several proteins which show change in expression in PTEN containing cells, study of these groups or networks in this context can throw more light on PTEN functions and targets for therapy in PTEN mutated tumors.

References

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Acknowledgement

This work was supported by kind grant from CERN foundation