Absolute quantification of key pathway proteins reveals SOS1 as the potential bottleneck of ERK response in the Ras/MAPK pathway

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Overview

- Sensitive PRISM-SRM assay enables for reliable quantification of proteins at ≤100 copies per cell.
- Absolute quantification data allow comparison of protein abundances within a single cell line as well as across multiple cell lines.
- Absolute quantification of pathway proteins opens a new avenue for revealing key regulators in the cellular signaling networks.

Introduction

- Generation of mathematical models for accurate prediction of cellular responses requires accurate absolute abundance data as well as dynamic data.
- The absolute abundance data is extremely scarce due to the lack of sensitive, reproducible, multiplexed protein quantification technologies.
- Selected reaction monitoring (SRM)-based targeted proteomics has been emerged as a promising technology; however, its sensitivity is often a limitation.[1]
- We recently achieved a breakthrough in SRM sensitivity (≥200 fold enhancement) by developing an antibody-free PRISM technique for effectively enriching target peptides with nearly lossless recovery.[2-7]

Methods

PRISM-SRM[2]

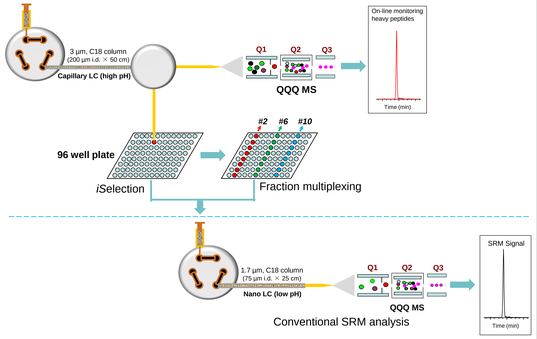

Figure 1. PRISM-SRM workflow for sensitive protein quantification.

Absolute quantification of pathway proteins

- 8 human cell lines (4 biological replicates for each cell line): BT20, HMEC, MCF10A, MCF7, NHDF, MDA-MB231, SKBR3, HS578T.
- 32 key EGFR pathway proteins were selected, which belong to two subcellular signaling pathways, the Ras/MAPK pathway and the PI3K/Akt pathway.

Phospho-ERK ELISA analysis

- To determine pERK sensitivity, EGF was added directly to culture plates to achieve the required doses (0.01, 0.1, 0.32, 1.0, 3.2, 10.0, 32.0, 100.0 ng/mL) for 10 min.
- Each dose point has three biological replicates and 7 human cell lines (BT20, HMEC, MCF10A, MCF7, MDA-MB231, SKBR3, HS578T) were treated with different doses of EGF with a total of 168 samples.
- The ERK phosphorylation levels at the EGF-stimulated human cells were determined using phospho-ERK ELISA assay, which was performed at Harvard Medical School.

Results

Sensitive quantification of Ras/MAPK pathway proteins by PRISM-SRM

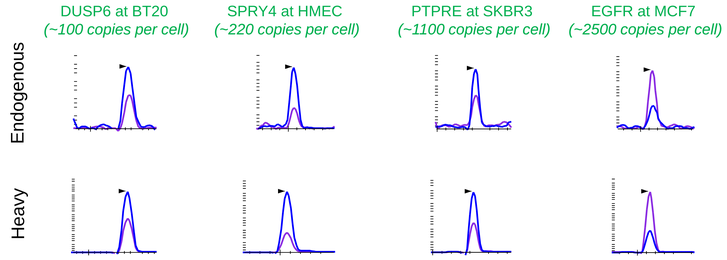

Figure 2. Sensitive detection and quantification of extremely low-abundance proteins by PRISM-SRM.

mRNA-protein correlations

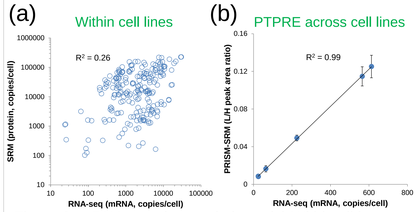

Figure 3. mRNA-protein correlations: (a) Within all human cell lines; (b) PTPRE across all human cell lines.

Strong correlation between pERK sensitivity and SOS1 abundance

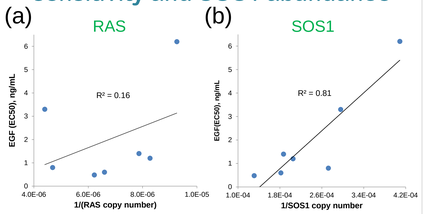

Figure 4. Correlations between the pERK sensitivity and absolute protein abundance: (a) H/K/NRAS; (b) SOS1.

SOS1 is the bottleneck due to its lowest concentration for all human cells

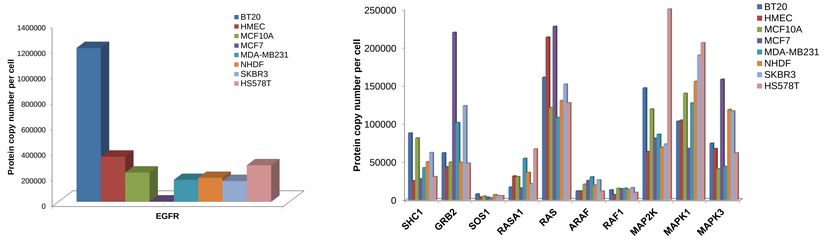

Figure 5. Comparisons of absolute abundance of core Ras/MAPK pathway proteins within all 8 human cell lines.

Conclusions

- PRISM-SRM enables reliable quantification of extremely low-abundance feedback regulators at ≤100 copies per human cell.
- Comparative analysis of mRNA and protein abundance levels revealed that there were significant correlations across cell lines, but there are very weak correlations within a single cell line.
- Comparisons of absolute abundance of core Ras/MAPK pathway proteins indicated that SOS1 has the lowest concentration for all human cell lines. This suggests that SOS1 is the potential bottleneck to determine the concentration of core Ras/MAPK protein complex, which is proportional to pERK response.
- This conclusion was confirmed by significant correlation of SOS1 abundance with the pERK sensitivity while no correlations for the other core Ras/MAPK pathway proteins.

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