

Global and Targeted Quantification of Seven Human Cell Lines Reveals the Correlation of Cell Type-Specific Responses with Feedback Regulators

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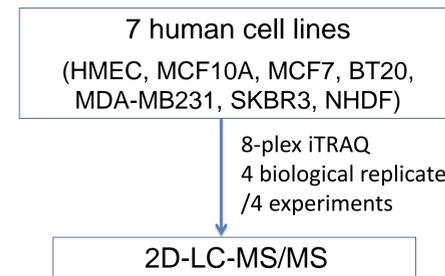
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Introduction

- Signal transduction networks are fundamental to regulating the complex behavior of cells as they underlay the coordination of multiple processes crucial to tissue function.
- One of the most extensively studied model systems over the last several decades is the ERK/MAPK pathway, which exhibits complex and diverse dynamics.
- Because of its critical role in regulating cell proliferation, understanding ERK regulation is central to efforts in rationally designing new antiproliferative drugs and other therapies.
- The epidermal growth factor receptor (EGFR) system is one of the most evolutionarily conserved signaling pathways for ERK activation, yet different cell types show distinct differences in their responses.
- Herein we applied global and targeted quantification to determine the basis of cell-specific differences in ERK responses based on protein expression levels across seven human mammary cell lines.
- We observed that the abundances of most core pathway proteins except EGFR were displayed at similar levels across the human cell lines. However, transcriptionally controlled feedback regulators were all expressed at low levels but displayed highly variable abundances between cell types.

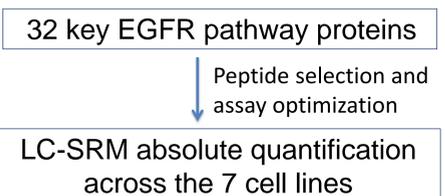
Methods

Global profiling



- Each 8-plex labeled sample was fractionated by high pH reversed phase chromatography into 24 fractions
- Fractions were analyzed by LC-MS/MS on a Velos Orbitrap mass spectrometer

Targeted quantification



- 4-5 crude peptides were purchased for each protein in initial assay development
- 2 peptides per protein were used for final analysis; one purified peptide was used for absolute quantification
- PRISM-SRM was used for quantification of low-abundance proteins¹

ERK response measurement

- Phosphorylated ERK level was measured by p-ERK immunoassay at Peter Sorger's Lab
- pERK levels were measured in a dose dependent format as stimulated by epidermal growth factor (EGF)

Results

Variable protein abundances across cell lines

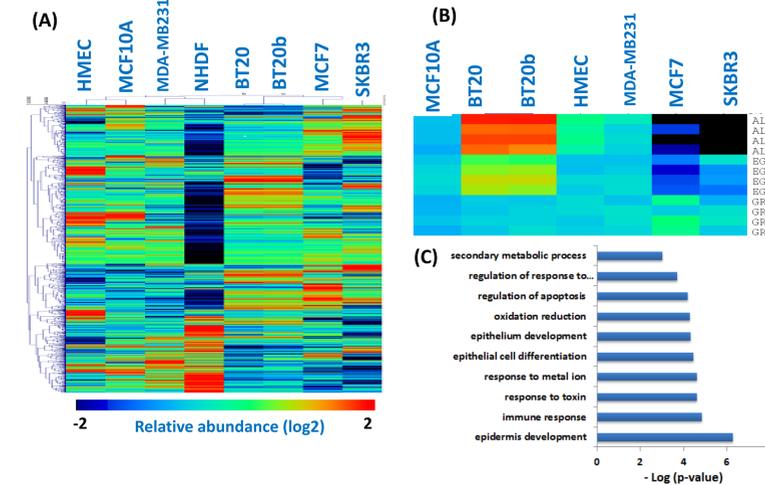


Figure 1. Protein abundance variations across 7 cell lines as measured by global proteomics (two technical replicates for BT20). (A) Heatmap of ~460 highly variable proteins. A total of ~3000 proteins were quantified. (B) Heatmap of selected proteins showing relatively good reproducibility across biological replicates. (C) Enriched biological processes for highly variable proteins.

Absolute quantification of EGFR network

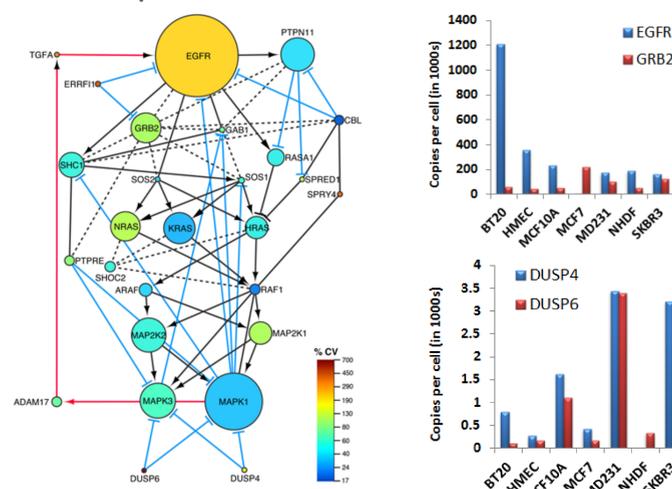


Figure 2. Absolute quantification of 32 key EGFR pathway proteins. Left: EGFR signaling network with protein node sizes corresponding to their abundances. Right: absolute abundances for selected proteins.

Global versus targeted quantification

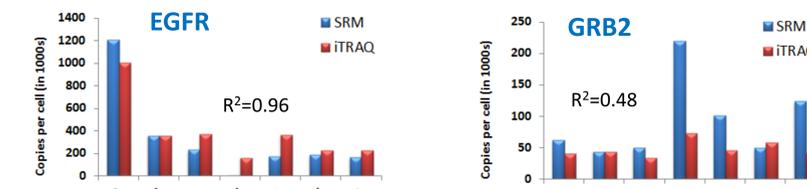


Figure 3. Comparison between iTRAQ-based global and SRM-based targeted quantification. Absolute abundances of iTRAQ data were calculated based on the copy number of HMEC and iTRAQ ratios

Correlation of protein abundances with pERK responses

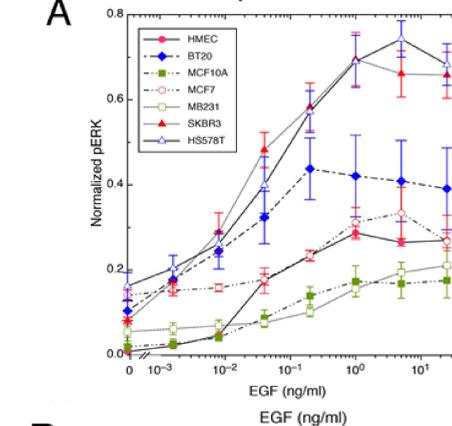
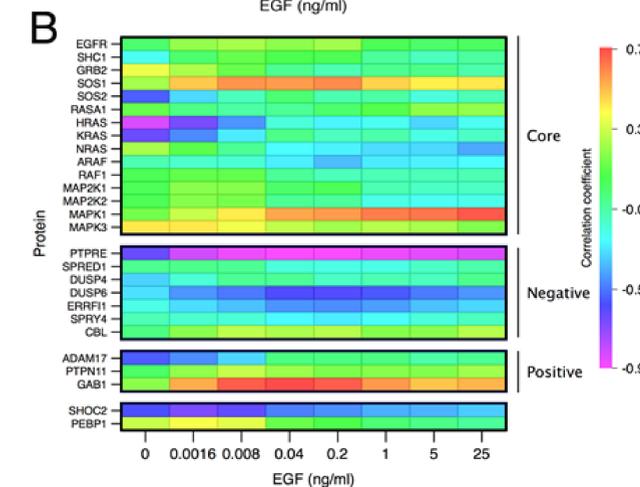


Figure 4. Relationship between response of a panel of cells to EGF and abundance of EGFR-ERK pathway proteins. (A) Response of panel of cells to varying EGF concentrations for 10 min prior to fixation and evaluation of pERK levels by immunofluorescence. (B) Correlation between normalized pERK levels at different EGF doses and abundance of specific EGFR-ERK pathway proteins. Pearson's correlation coefficients were calculated.



Conclusions

- Global proteomics quantified ~3,000 proteins across 7 cell lines. However, it still lacks sensitivity for low-abundance pathway proteins in that only 11 out of 32 EGFR proteins were reliably detected.
- Ultrasensitive PRISM-SRM allows accurate measurements of all 32 EGFR pathway proteins across 7 cell lines.
- The advantages of targeted quantification in terms of sensitivity, accuracy, and dynamic range were clearly demonstrated.
- Among the 32 proteins, the stoichiometric differences can be >1000-fold (e.g., EGFR vs. DUSP6).
- Differential ERK responses were observed to correlate most strongly with the abundances of several feedback regulators, which suggests that cell-specific expression of feedback regulators of the EGFR pathway is most likely responsible for the cell type specific differences observed in ERK activation.

Acknowledgements

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