A carrier-assisted targeted mass spectrometry approach for proteomics analysis of single cells

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Overview

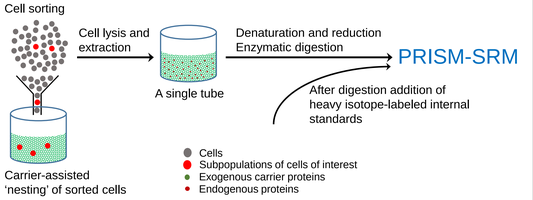
- A simple, convenient targeted MS approach, cPRISM-SRM, was developed for enabling highly sensitive quantification of proteins in single cells.
- Proteins with ≥25,000 copies/cell in 10 mammalian cells can be reliably detected by cPRISM-SRM with minimal sample loss.
- cPRISM-SRM opens a new avenue in biomedical research involving single cells or extremely small amounts of precious clinical specimens.

Introduction

- Antibody-based flow cytometry and mass cytometry are predominant technologies for targeted proteomics analysis of single cells.
- Cytometry methods share common shortcomings with other antibody-based methods (e.g., low-multiplex, the need of high-quality antibodies, and unavailability of antibodies for new proteins), as well as lack quantitation accuracy to provide accurate protein concentrations.[1,2]
- MS-based targeted proteomics has emerged as a promising alternative for antibody-free, precise, high-multiplex quantification of target proteins.[3]
- There is an unmet challenge for MS-based proteomics analysis of single cells because of ineffective sampling and insufficient MS sensitivity.[4]

Methods

cPRISM-SRM



PRISM-SRM[5,6]

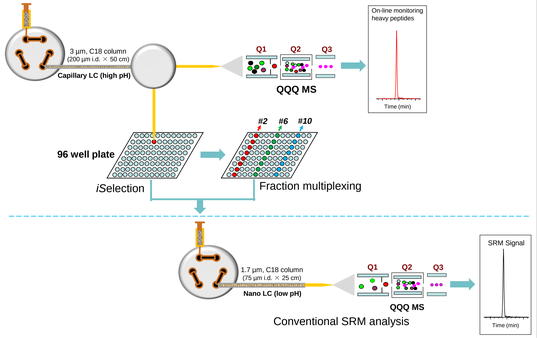


Figure 1. cPRISM-SRM workflow for proteomics analysis of single cells. Exogenous BSA protein was used as a carrier for lossless processing of single cells. A highly sensitive targeted MS platform PRISM-SRM was then used for absolute quantification of target proteins.

Two types of a small number of cells

- Mimic cells: an equivalent small number of cells from large HMEC digests spiked into ~25 µg of BSA digests.
- Intact cells: a small number of intact HMEC cells (from serial dilution) collected into a tube containing 50 µg of BSA followed by normal sample processing.

Results

Mimic HMEC cells

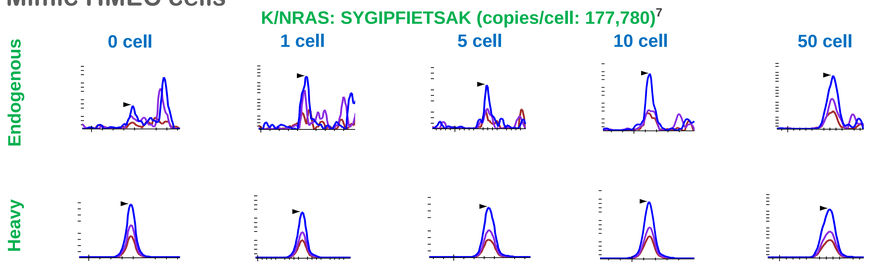


Figure 2. Extracted ion chromatograms (XICs) of transitions monitored by cPRISM-SRM at various numbers of cells (1-1000 cells).

Calibration curves

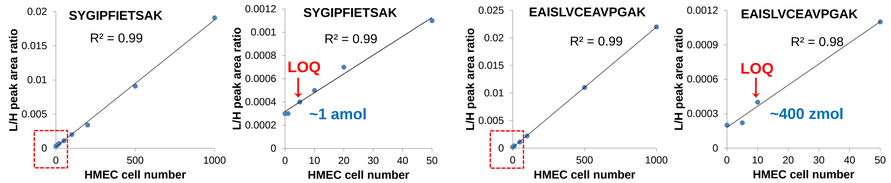


Figure 3. Calibration curves for quantification of K/NRAS (SYGIPFIETSAK) and SHC1 (EAISLVCEAVPGAK) at 1-1000 cells.

Intact HMEC cells (10 *vs* 100 cells)

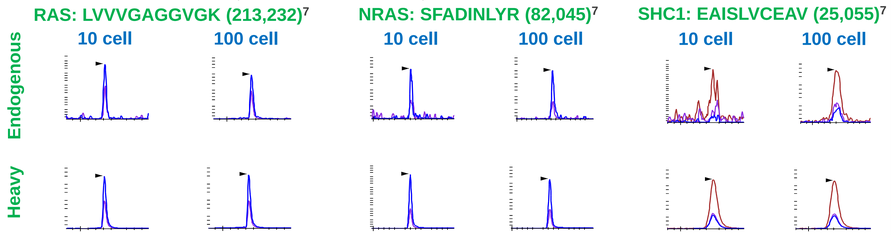


Figure 4. XICs of transitions monitored by cPRISM-SRM at 10 and 100 intact HMEC cells isolated from serial dilution.

Conclusions

- We developed a new targeted MS approach, cPRISM-SRM, that couples carrier-assisted sample preparation (to address ineffective sampling) to a highly sensitive targeted MS platform (to address MS sensitivity issue) for enabling proteomics analysis of single cells.
- cPRISM-SRM was demonstrated for reliable quantification of EGFR pathway proteins at ≥25,000 copies/human cell in single cells (e.g., 10 HMECs).
- We envision that this new targeted MS capability will have broad utility in biomedical research for absolute protein quantification in small-size samples that are not readily accessible by current proteomics techniques (e.g., small subpopulations of cancer cells and circulating tumor cells).

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