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

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Methods

cPRISM-SRM

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.

• Cytochemistry methods share common shortcomings with other antibody-based methods (e.g., low-multiplex, the need for high-quality antibodies, and unavailability of antibodies for new proteins), as well as lack quantitation accuracy to provide accurate protein concentrations.

• MS-based targeted proteomics has emerged as a promising alternative for antibody-free, precise, high-multiplex quantification of target proteins.

• There is an urgent challenge for MS-based proteomics analysis of single cells because of ineffective sampling and insufficient MS sensitivity.

Results

Mimic HMEC cells

KRAS: SYGGFIETSASK (copies/cell: 177,780)7

Intact HMEC cells (10 vs 100 cells)

Calibration curves

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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