DETECTION OF SINGLE-CELL ENZYME ACTIVITY BY SINGLE-TIME-POINTSTABLE ISOTOPE PROBING-MASS SPECTROMETRY

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Abstract - Elucidation of single-cell enzyme activity is essential for clarifying cellular heterogeneity and its causal relationship with cell fate. In the currently available methods for enzyme activity detection, the traditional multiple-time-point approaches using the Michaelis-Menten model are susceptible to prolonged measurement periods and thus compromise normal cellular functions, whereas the single-time-point approaches using Line weaver-Burk plots are limited by sample inequality and difficulty of aliquoting a single-cell sample. Mass spectrometry (MS) has the attractive advantage of simultaneous detection of multiple molecular components. Among the MS-based assays, stable isotope probing (SIP)-MS has provided an idea of single-cell enzyme activity analysis using a single-time-point approach. In this study, a single-time-point SIP-MS assay was developed for revealing enzyme activity heterogeneity in breast cancer single cells. Specifically, a series of stable isotope labelled substrate peptides were pooled to estimate the activity of target enzyme Cathepsin D (CTSD). The results indicated the cellular heterogeneity of CTSD activity and the positive correlation between CTSD activity and the metastatic capability of cells. Furthermore, the fusion of cancer cells with M2 macrophages, as a representative cellular event in cancer development, was monitored using the developed single-time-point SIP-MS assay. Owing to their greater metastatic potential, the fused cells could be well distinguished from the unfused cells based on the detected CTSD activity at the single-cell level.

Keywords - Single-Cell Enzyme Activity Assay, Stable Isotope Probing-Mass spectrometry(SIP-MS), Single-Time-Point Detection, Cathepsin D (CTSD), Breast cancer Metastasis