National Taiwan University

TP 491: Single-cell lipidomics by nanoflow liquid chromatography combined with multinozzle emitter mass spectrometer

When: Tuesday, June 7th; 10:30am – 2:30pm CT

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Introduction

Cell heterogeneity in tumors may lead poor chemotherapy efficacy, metastasis, and recurrence. Single-cell lipidomics provides a powerful tool to reveal the phenotypic differences to investigate the cell heterogeneity at chemistry level. The changes of lipid profiles in tumor cells are known and mass spectrometry (MS) has been widely used for quantification and characterization of cellular lipids. However, current single-cell analysis methods, such as direct infusion electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI) MS, usually encounter serious ion suppression effect. Hence, we propose to establish a high sensitivity platform for single cell analysis via nanoflow liquid chromatography with multinozzle emitter ESI MS for a deeper lipidomic profiling.

Methods

The single cell-analysis platform established by nanoAcquity UPLC™ system (Waters MS Technologies, Manchester, UK). The 1 μL sample was direct injected onto the analytical column by flowrate was 350 nLmL -1 and total run tine was 110 minutes, and coupled to a Q-Exactive Plus hybrid quadrupole-orbitrap mass spectrometer (ThermoFisher Scientific) operated in scheduled Top10 data-dependent acquisition.In order to obtain a lower limit of detection, we used the multinozzle emitter system- Newomics® M3 Emitter (10 um)-8 Nozzles to generate electrospray ionization.The performance was compared with Thermo Scientific SS Emitters via three phospholipids standards calibration curve.We collected osteosarcoma single cell by FACS (BD FACSAria III) and stored in 96 well plate with 50 ul PBS at -80 oC.

Preliminary Data

In general, the sensitivity of the multinozzle source was better than the single nozzle emitter in term of lipid detection. For example, the limit of detections (LODs) for phosphatidylcholines (PC) (36:2) standard was 0.01 ng mL -1 on the multinozzle emitter and 0.05 ng mL -1 on single nozzle emitter. Based on this result, we proceeded to obtain lipidomic profiles of osteosarcoma single cell through the multinozzle emitter coupling with nanoflow UPLC. To discriminate the lipid signal of single cells from blank noise signal, statistical analysis was carried out. The volcano plot from single cell and matrix blank indicated that 144 ion species were found to have significantly increased osteosarcoma single cells (N=3, $p < 0.05$, log 2 Fold Change >1). In addition, we analyzed 500-cell extract to establish the lipid database via LipidSearch software, and compared the precursor ion (MS1) and retention time of the 500-cell extract with osteosarcoma single cells. Further analysis of osteosarcoma single-cell samples identified 18 lipids, including phosphatidylcholines (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidic acid (PA), ceramides (Cer), Sphingomyelin (SM) and triglycerides (TG). Next, the absolute amount of PC 36:2 in an

osteosarcoma single cell was determined to be ~ 6.7 picogram per cell via standard calibration curve. With the preliminary date provided in this work we believe a great potential of our platform for nano-to-micro analysis of single cell lipidomics.

Novel Aspect

We successfully establish a single-cell lipidomics analysis platform via nanoflow liquid chromatography combined with high-resolution mass spectrometer.