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Near Single-Cell Proteomics (nscProteomics)

Abstract: This novel technology utilizes recently developed nanoscale proteomics analysis capability in which a few cells can be processed to generate cell-specific protein profile. This technology has two major components. The first component includes sectioning of tissue section on a glass slide with or without cell specific stain. The stained sections are used to guide laser capture of cells of interest using laser capture microdissection device. Second component of the technology includes proteomics sample preparation and nanoliter scale and mass analysis of protein sample at nanoliter scale. This technology brings value to KPMP by mapping cell specific proteins to the map generated by image and transcriptome analysis. Having a clear understanding of thousands of expressed proteins at cellular level from robust mass spectrometric analysis provides a better and more granular view of the cells compared to imaging methods, which are usually low throughput and suffer from the specificity antibodies to their corresponding antigens.

Detailed Procedure

[A] Laser Capture Microdissection of the cells of interest

1. Kidney sections were cut from OCT (optimal cutting temperature compound) blocks using a cryo-microtome and mounted on PET slides (cat no. 415190-9051-000, Zeiss)
2. Slides were briefly fixed with methanol, rinsed with H_2O to remove OCT, and dehydrated using an alcohol gradient before placing in a desiccator to dry (8 min).
3. Two 10 um serial sections (OCT frozen) are mounted on Zeiss PET MembraneSlide.
4. Use one slide to stain with appropriate stain to localize the cells of interest
5. Use the second slide top use capturing of the cells of interest using Zeiss PALM Laser Microdissection system using AdhesiveCap 200 opaque (cat no. 415190-9181-000, Zeiss)

[B] Application of nanoPOTS technology for protein identification and quantification

6. Use customized nanoPOTS technology [1] to prepare samples for nscProteomics.
   a) For this, use deionized water (18.2 MΩ) purified using a Barnstead Nanopure Infinity system (Los Angeles, CA, USA). DTT and iodoacetamide (IAA) from Thermo Scientific (St. Louis, MO, USA) and freshly prepared in 50 mM ammonium bicarbonate buffer each day before use. RapiGest SF surfactant (Waters, Milford, MA, USA) dissolved in 50 mM ammonium bicarbonate buffer with a concentration of 0.2% (w/w), aliquoted, and stored at −20°C until use. Trypsin (MS grade) and Lys-C (MS grade) from Promega (Madison, WI, USA).
   b) Use accustom built nanowell chip with an array of 3 × 7 spots with diameters of 1 mm and a spacing of 4.5 mm on a 25 mm × 75 mm glass slide that was pre-coated with chromium and photoresist.and a glass spacer (25 mm × 75 mm × 1 mm).
   c) Use a capillary probe 200 µm i.d., 360 µm o.d., with a tapered tip (30 µm i.d., 50 µm o.d.) for liquid dispensing of the syringe pump.
   d) Use a liquid handling system with a metering precision of 0.3 nL.
7. Use customized SPE-LC-MS for mass spectrometry data generation using Orbitrap Fusion Lumos Tribrid MS (ThermoFisher).

8. Use Maxquant (version 1.5.3.30) to process raw files for feature detection, database searching, and protein/peptide quantification against UniProtKB/Swiss-Prot human database.

QA/QC parameters

1. Collect >20 cells of interest for each patient biopsy
2. The OrbiTrap Fusion mass spectrometer is subjected to a mass calibration every week to ensure the instrument is optimized for obtaining high mass accuracy (< 3 ppm) data.
3. The mass spectrometer is subjected to full calibration monthly to ensure that not only high mass accuracy is obtained, but to also ensure high sensitivity is obtained.
4. HPLC function along with the mass spectrometer's mass accuracy and resolution are tested by injecting a five-peptide standard (500 fmol of each peptide) into the LC-MS/MS system.
5. HPLC retention times for each peptide should not deviate more than one minute from historic norms, mass accuracy should be less than 3 ppm, and mass resolution should be better than 120,000 at m/z 200.
6. The minimum peptide length is 7 amino acids and maximum peptide mass was 4600 Da.
7. Missed cleavages for each peptide = 2.
8. For both peptides and proteins maximum false discovery rate (FDR) of 0.01

References:

[1] Zhu Y, Piehowski PD, Zhao R, Chen J, Shen Y, Moore RJ, Shukla AK, Petyuk VA, Campbell-Thompson M, Mathews CE, Smith RD, Qian WJ, Kelly RT. Nanodroplet processing platform for deep and quantitative proteome profiling of 10-100 mammalian cells. Nat Commun. 2018 Feb 28;9(1):882. PubMed PMID: 29491378