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Workflow for human placental ECM proteomics

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Described here is the workflow used by the Female Reproductive Tissue Mapping Center at UCSD to generate extracellular matrix proteomics (ECM) data from human placenta.

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Described here is the workflow used by the Female Reproductive Tissue Mapping Center at UCSD to generate extracellular matrix proteomics (ECM) data from human placenta.

Extracellular Matrix (ECM) proteomics for the UCSD Female Reproductive TMC Summary of Procedures

Prepare tissue according to the following protocol:
Human Placenta Tissue Collection and Preservation Methods - UCSD Female Reproductive TMC
For this protocol, use tissue that has been snap-frozen.

I. Sample Extraction and Digestion:

1. Lyophilized samples were weighed (~5mgs), and brought up in 50mM Tris-HCl (pH 7.4), .25% CHAPS, 25mM EDTA, 3M NaCl supplemented with 10ul/mL Halt Protease Arrest.
2. Approximately 100 mg of 3mm glass beads were used to mechanically agitate samples in a Bullet Blender
3. Vortexed @ 4°C for 20 min
4. Centrifuged at 18,000 x g for 20 minutes, supernatant removed
5. Steps b-d repeated x 2
6. Pellets subjected to chemical digestion with 1M hydroxylamine hydrochloride (HA) in 6M Gnd-HCl
7. Protein concentration of each sample determined by BCA
8. Proteolytic digestion was carried out according to the FASP protocol[1] with 10 kDa molecular weight cutoff filters (Sartorius Vivacon 500 #VN01H02) using 30 ug of protein resulting from each sample.
9. Samples reduced with tris(2-carboxyethyl)phosphine), alkylated with 2-chloroacetamide, and digested with trypsin (1:100) at 37ºC for 14 Hrs. Peptides were recovered from the filter using successive washes with 0.2% formic acid.

II.LC-MS/MS:

1. Resulting peptides were analyzed by LC-MS/MS using a Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific) coupled to an EASY-nLC 1200 (Thermo Fisher Scientific) operating in data dependent acquisition mode.
2. Raw spectra were interpreted against the human proteome database using the MSFragger-based FragPipe computational platform[2]

Methods

Sample preparation for LC-MS/MS

Lyophilized samples were weighed (~5mgs) and brought up in 50mM Tris-HCl (pH 7.4), .25% CHAPS, 25mM EDTA, 3M NaCl supplemented with 10ul/mL Halt Protease Arrest. Approximately 100 mg of 3mm glass beads were used to mechanically agitate samples in a Bullet Blender (NextAdvance) prior to all cellular and ECM extraction steps. Protein concentration of each fraction for each sample was measured using A660 Protein Assay (Pierce™). Proteolytic digestion was carried out according to the FASP protocol[1] with 10 kDa molecular weight cutoff filters (Sartorius Vivacon 500 #VN01H02) using 30 ug of protein resulting from each fraction. Samples reduced with tris(2-carboxyethyl)phosphine), alkylated with 2-chloroacetamide, and digested with trypsin (1:100) at 37ºC for 14 hrs. Peptides were recovered from the filter using successive washes with 0.2% formic acid. Peptides were recovered from the filter using successive washes with 0.2% formic acid.

LC-MS/MS analysis

Global proteomics for all comparative method testing was carried out on a Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific) coupled to an EASY-nLC 1200 (Thermo Fisher Scientific) through a nanoelectrospray LC – MS interface. Eight µL of each sample was injected into a 20 µL loop using the autosampler. The analytical column was then switched on-line at 400 nl/min

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over an in house-made 100 µm i.d. × 150 mm fused silica capillary packed with 2.7 µm CORTECS C18 resin (Waters; Milford, MA). LC mobile phase solvents consisted of 0.1% formic acid in water (Buffer A) and 0.1% formic acid in 80% acetonitrile (Buffer B, Optima™ LC/MS, Fisher Scientific, Pittsburgh, PA). After 22 uL of sample loading at a maximum column pressure of 700 bar, each sample was separated on a 120-min gradient at a constant flow rate of 400 nL/min. The separation gradient for cell fractions consisted of 6% buffer B from 0 to 3 minutes, followed by a linear gradient from 6 to 42% buffer B from 3 minutes to 105 minutes. Linear gradients from 6 to 36% and 6 to 24% buffer B were utilized from 3 to 105 minutes for the sECM and iECM fractions, respectively. Gradient elution was followed by a linear increase to 55% buffer B from 105 to 110 minutes and further to 95% buffer B from 110 to 111 minutes. Flow at 95% buffer B was maintained from 111 minutes to 120 minutes to remove remaining peptides. Data acquisition was performed using the instrument supplied Xcalibur™ (version 4.5) software. The mass spectrometer was operated in the positive ion mode. Each survey scan of m/z 375–1600 was followed by higher energy collisional dissociation (HCD) MS/MS (30% collision energy) using the standard AGC target and a 35 ms maximum injection time with an isolation width of 1.6 m/z. The orbitrap was used for MS1 and MS2 detection at resolutions of 120,000 and 50,000, respectively. Dynamic exclusion was performed after fragmenting a precursor 1 time for a duration of 45 sec. Singly charged ions were excluded from HCD selection.

Data processing

Fragmentation spectra were searched against the UniProt humanproteome database using the MSFragger-based FragPipe computational platform[2]. Contaminants and reverse decoys and were added to the database automatically. The precursor-ion mass tolerance and fragment-ion mass tolerance were set to 10 ppm and .2 Da, respectively. Precursor tolerance was set to ±10 ppm and fragment tolerance was set to ±0.04 Da, allowing for 2 missed cleavages. Fixed modifications were set as carbamidomethyl (C). Variable modifications were set as oxidation (M), oxidation (P) (hydroxyproline), Gln->pyro-Glu (N-term Q), deamidated (NQ), and acetyl (Protein N-term). Results were filtered to 1% FDR at the peptide and protein level.

[1]Wiśniewski, J.R., 2016. Quantitative evaluation of filter aided sample preparation (FASP) and multienzyme digestion FASP protocols. *Analytical chemistry*, *88*(10), pp.5438-5443.

[2]Kong, A.T., Leprevost, F.V., Avtonomov, D.M., Mellacheruvu, D. and Nesvizhskii, A.I., 2017. MSFragger: ultrafast and comprehensive peptide identification in mass spectrometry–based proteomics. *Nature methods*, *14*(5), pp.513-520.

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