IPD Project Details

Project ID: IPD1891

Project Title: Deep proteome analysis of more than 12000 proteins in buffalo mammary epithelial cells identifies protein signatures for active proliferation and lactation

Description: Extensive branching morphogenesis takes place during pregnancy in the mammary gland. It is accompanied by the rapid proliferation of the Mammary Epithelial Cells (MECs). To gain insights into the proteomic changes that occur during the proliferation of MECs from buffalo (Bubalus bubalis) origin, we explored the deep proteome profile of buffalo mammary epithelial cells (BuMECs) using mass spectrometry (MS). To achieve this, we employed the sub-cellular fractionation approach and secretome analysis. Proteins were isolated separately from four subcellular fractions (SCFs) containing cytosolic (SCF-I), membranous and membranous organelleâ€™s (SCF-II), nuclear (SCF-III) and cytoskeletal (SCF-IV). These sub-cellular specific protein fractions were processed using in-solution digestion and analyzed with nano-LCMS/MS. The MS analysis identified 8330, 5970, 5288 and 4818 non-redundant proteins in the fractions SCF I, II, III and IV respectively. To evaluate the secretory proteins in these cells, gel-based proteome approach was used which revealed a total of 792 non-redundant proteins. Altogether, combined analysis of all the five fractions including four sub-cellular fractions and secretome resulted in the identification of 12,609 non-redundant proteins. A total of 325 molecular pathways were identified after extensive analysis. The most enriched pathways associated with these proteins were metabolic, PI3-AKT, MAPK, mTOR, Insulin, estrogen and Oxytocin signaling. Our study demonstrated for the first time highest number of proteins identified by MS in any cell types including MECs.

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Sample Preparation: The proteins were isolated from the four sub cellular fractions and the interfering substances such as detergents, salts, lipids and nucleic acids were removed from the protein preparations using 2D-clean up kit (GE Healthcare, USA). The pellet was rehydrated in the 2% Diethylamine and the total protein concentration was estimated using 2D-Quant kit (GE Healthcare, USA) as per manufacturer’s instructions. For the secretome analysis, the 30 ml conditioning media was collected from the

proliferating cells, filtered using 0.2 ?m syringe filter and centrifuged at 1500 rpm to remove the cell debris. Further, the media was concentrated using 3 kDa molecular weight cut off centricon at 4000 g and 4 °C, and stored at -80 °C until further processing for mass spectrometry.

Peptide Separation: The subcellular protein fractions were processed separately for the In-sol trypsin digestion. In brief, 45 mM DTT in 50 mM NH4HCO3 was used to reduce the disulfide bonds followed by alkylation of cysteine residues using 100 mM IAA in 50 mM NH4HCO3. Whereas, the conditioning media protein was digested using the In-gel method. In brief, destained bands were reduced with the 5 mMdithiothrietol (DTT) in 40 mM NH4HCO3. Digestion was carried out overnight using 12.5 ng/ul trypsin (Modified sequencing grade; Promega, USA) at 37 °C. Subsequently digested proteins were lyophilized and desalted using zip-tip C18 (Millipore, Germany) following manufacturer's instructions and stored at -80°C until MS analysis.

Protein Characterization: The nano-LCMS/MS results were analyzed using the Protein Scape software (McHugh et al., 2008 and Naru et al., 2016). Peak lists were generated by qTOF control (version 24.8) using the Hystar post processing program to automatically subtract baseline, smoothen peaks and to generate centroid data before submission to Mascot (2.4.1 Matrix Science, UK) with proteins identified by correlation of mass spectra to entries in the Bos taurus database. Mascot MS/MS ion search criteria were as follows: taxonomy-other mammalia, trypsin digestion, allowing up to one missed cleavage, peptide tolerance of 50 ppm, and MS/MS tolerance of 0.05 Da. The Carbamidomethylation of cysteine was searched as a fixed modification, whereas Carbamidomethyl, Gln->pyro-Glu, Carbamyl, Carboxymethyl, Oxidation, Phospho, and Glu -> pyro-Glu were searched as the variable modifications. The "ion score cutoff" was manually set to 15 thereby eliminating the lowest quality matches. To eliminate false positives, 1% FDR was applied at both protein and peptide levels. Subsequent bioinformatics analysis was performed using the various softwares including PANTHER (Protein Analysis through Evolutionary Relationships://www.pantherdb.org), DAVID v. 6.7, STRING (http://string-db.org) and Cytoscape plug-in ClueGo.

Experiment Type: Shotgun proteomics

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, phosphorylated residue, carbamoylated residue, iodoacetic acid derivatized residue, iodoacetamide derivatized residue

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