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Profiling of cell surface proteome and metabolome from an *In vitro* model system, using LC-MS technologies

Ravi K Krovidi[1], Arun Kumar Padmanaban[1], Lynette Lincoln[1], Syed Salman Lateef[1] and Leo Bonilla[2]
[1]Agilent Technologies India Pvt. Ltd., India
[2]Agilent Technologies, USA

Introduction: Cell surface membrane proteins play a predominant role in cellular signaling processes. Membrane specific receptor proteins serve as cellular markers, prime drug targets to several pharmaceutical agents. Receptor proteins have been targeted to decipher the molecular mechanisms for several cancers e.g.; breast, epidermoid and lung cancers. Immuno-phenotyping assays provide a comprehensive understanding of cell surface markers, but limited by the availability of sp. antibodies. We adopted the chemical biotinylation enrichment methodology coupled with LC-MS technologies to analyze the spatio-temporal changes of protein expression along with secreted metabolome signatures from EGF induced epidermoid carcinoma cell lines-A431. In our current study we evaluate the global qualitative profiling of cell surface proteins and secreted metabolites.

Methods: A431 cells were cultured in DMEM medium as described. Chemical biotinylation was carried out following the recommendations from Pierce chemicals. Briefly, the cells were grown to 90% confluence and washed with ice-cold PBS and Sulfo-NHS-SS-Biotin solution was added at a final concentration of 0.25 mg/ml for 30 minutes with gentle agitation. The adherent monolayer was washed, scrapped into PBS solution and centrifuged to collect the cell pellet, followed by lysing under denaturing condition, biotinylated proteins are then affinity purified using NeutrAvidin column with DTT denaturation. The eluted protein was digested with Trypsin/Lys-C mix, resulting peptides were analyzed using a microfluidic-based nanoflow LC coupled to Q-TOF MS. Data reprocessing was performed using software for protein database search for protein analysis.

Preliminary data: Employing the chemical enrichment strategy with NHS-SS-Biotin probe, target cell surface proteins were enriched from A-431, epidermoid carcinoma cell lines. Enriched proteins were subjected to proteolytic digestion using Trypsin/Lys-C mix. The resulting peptide mixtures were desalted and subjected to a medium to long 45 min linear gradient separations of acetonitrile (ACN) in 0.1% formic acid delivered at 300 nL/min over a C18 reverse phase LC system using a microfluidic device. LC-MS/MS data was acquired in both centroid and profile modes. Acquired spectra were then searched with Spectrum Mill search engine against the Homo sapiens, Uniprot FASTA protein database. A mass accuracy of +/- 50 ppm was used for precursor ions and 0.6 Da for product ions. Higher sensitivity levels of peptide detection with dual-stage ion funnel technology have resulted in the identification of several cell surface membrane proteins including extra cellular matrix proteins, moderately abundant proteins including pancreatic marker protein, Plectin-1 along with F-Box Leucine rich repeat protein-2, Beta Actin, PGK-2, Annexin-2, etc. Our preliminary results demonstrate the ability of the chemical affinity, LC-MS enrichment strategy, to identify the cell surface membrane proteins.

Cell surface proteome complemented with secreted metabolome from EGF induced epidermoid carcinoma cell lines would provide a deeper understanding of the spatio-temporal events of the cellular machinery and an insight into potential metabolite signatures upon drug treatment. Metabolome profiling and data anlaysis is currently underway.

ravi_krovvidi@agilent.com