IPD Project Details

Project ID: IPD2981

Project Title: PROTEOMIC ALTERATIONS IN COLON ADENOCARCINOMA TISSUE LYSATES

Description: Colorectal cancer is the third most common cancer worldwide, with 1.4 million people diagnosed in 2012 according to GLOBOCAN 2012. 95% of the colon cancer cases are that of adenocarcinomas. Removal of high-risk adenomas and tumors at early stage of colorectal cancer (CRC) can prevent its onset/progression into higher grades of cancer. The prognosis of colorectal cancer and the stage of diagnosis are closely correlated. 5-year survival rate is observed among 90% patients when diagnosed early and in less than 10% when the metastases develop. This makes the implementation of screening methods aimed at early detection paramount for reduced incidence and mortality rate. Adenocarcinomas are the cancers originating from the gland forming cells of the colon and rectal lining and are known to be the most common type of colorectal cancer. The current diagnosis options for colorectal cancers are limited to biopsy, stool tests and other laboratory tests, barium enema based imaging and other imaging techniques, colonoscopy and other endoscopic procedures which are time consuming. In this study, we used proteomics approach with an aim to identify protein biomarkers which can aid in early detection of colon adenocarcinomas to be precise. Proteins from tumor tissue of colon adenocarcinoma subjects (n=11) and their matched controls were subjected to 4-plex iTRAQ labelling followed by off-gel fractionation prior to LC-MS/MS run. The mass spectrometry data was analysed independently using two different analysis software - Spectrum Mill (SM) and Trans Proteome Pipeline (TPP). The proteins identified using either SM and/or TPP were subjected to pathway analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 and the proteins common between the two analyses were compared with the data from CPTAC portal and Human Protein Atlas. The expression level of the shortlisted panel of proteins was studied in brain cancers as a non-colon adenocarcinoma control group and validated using MRM approach. A list of 285 unique proteins was identified to be significantly dysregulated in colon adenocarcinoma as compared to its matched controls. These proteins were found to be involved in glycolysis, pentose phosphate pathway, biosynthesis of amino acids, protein processing, spliceosome, proteosome, focal adhesion and proteoglycans in cancer. 94 of the 285 proteins were identified by both-SM and TPP. 34 of these 94 proteins were found to be dysregulated with same trend as that in data reported on CPTAC portal and 9 of these 34 proteins were validated using MRM approach. The proteins identified from this study could be validated further to

investigate the role of these proteins as potential biomarkers for early detection of colon adenocarcinoma.

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Sample Preparation: 11 colon adenocarcinoma RIPA tissue lysates and their matched normal RIPA lysates were received from Department of Human Molecular Genetics & Biochemistry, Sackler Medical School, Tel Aviv University, Israel. Protein samples (in RIPA buffer) were exchanged to TEAB buffer using Amicon Ultra 0.5 mL centrifugal 3 kDa filters (Merck Millipore, Germany). After buffer exchange, quantification of protein content in each sample was performed using QuickStart Bradford reagent (BioRad, USA). Quantitative proteomics analysis was performed on control and tumor colon adenocarcinoma cohorts using iTRAQ labelling. Sample labeling strategy of four different sets for differential proteomic analysis was: each set had a pool of all the control tissue lysates (n=11) labelled with 114 and 3 of the individual tumor tissue lysates labelled with 115, 116 and 117. One of the set consisted of the pool of all the tumor tissue lysates (n=11) labelled with 117. Prior to the iTRAQ labeling, in-solution digestion was performed (75?g proteins from each sample) following the manufacturer's instructions.

Peptide Separation: After in-solution digestion, iTRAQ labeling of the peptides was performed following the manufacturer's instructions (AB Sciex, USA). OFFGEL fractionation of the labeled peptides was performed using a 3100 OFFGEL fractionator (Agilent Technologies, Santa Clara, CA) with high resolution (pH 3-10, 24 cm) IPG strips. Agilent 6550 iFunnel Q-TOF LC MS/MS instrument (Agilent Technologies, USA) equipped with a Chip-Cube controlled by the Mass hunter Acquisition software in a positive ion mode was applied for quantitative proteomic analysis. The range of the MS spectra was kept between 100-3200 and for MS/MS 50-3200. The instrument was operated using AutoMS/MS, in a data operated manner. Mass tolerance of 20 ppm and fragment mass tolerance of 50 ppm was specified.

Protein Characterization: Protein identification and quantification of the iTRAQ reporter ion intensities were performed using Spectrum Mill identification software (Agilent Technologies, USA). The Paragon algorithm was used as default method for search with trypsin as a digesting agent and IAA for cysteine and iTRAQ (N-term K) were selected for the fixed modification and oxidized methionine as the variable modification. Peptide mass spectra obtained were searched against the SwissProt database using Homosapiens as taxonomy. p-values obtained from a paired t-test were used to evaluate significance of differences in the protein abundances between normal and colon adenocarcinoma study cohorts. P-values (adjusted) < 0.05 were considered to be statistically significant.

Experiment Type: Shotgun proteomics

Species: Homo sapiens - 9606

Tissue: Colon (bto:0000269)

Cell Type: Epithelial cell (cl:0000066)

Disease: Colon cancer (doid:219)

Instrument Details: 6510 Quadrupole Time-of-Flight LC/MS (MS:1000676)

Protein Modifications: No PTMs

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