Disease proteomics

198: Serum profiling for candidate biomarkers: decrypting the chrono encryption

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The low molecular weight region of human serum has been identified as a potential source for biomarkers. The abundant secreted proteins in circulation tend to leave tell-tale fragment peptides which can be of use as surrogate biomarkers. Specifically proteases present in the clotting cascade are key functional components that contribute to this peptide pool. The magnetic bead based weak cation exchange technique (MBB) is a simple, automatable, highly-reproducible affinity purification technique custom built for the peptidome profiling. In our current study we evaluated the effect of potential clinical and sample handling variables that might skew the results of a clinical proteomics/peptidomics strategy. We utilized magnetic bead based weak cation exchange in a technique standardization sample set (SC) comprising exclusively of healthy individuals (n = 6) and a case/control (39 cases/42 controls) cohort of coronary artery disease (CAD). The SC cohort consisted of normal individuals whose serum samples were collected and serially analyzed for in vitro preanalytical variations due to, time since collection (archival time), temperature of storage and freeze thaw cycles. We found that, time since collection acts as a major pre-analytical variable while utilizing MBB for case control differentiation. The peaks have a time specific degradation pattern which interferes with the case control classification. The intensity of 11 peptide peaks were found to increase with archival time. We replicated these findings in six serum samples obtained from healthy volunteers. These samples were kept at −80°C and −20°C for about a year to ascertain the effect of archival time and temperature. Huge peptide profile changes were found when samples were kept at −20°C as compared to −80°C. Both storage temperature and time since collection was found to have a major impact on the serum protein profiling. We believe that designing of stringent protocols that reduce in vitro/ex vivo artifacts is the need of the hour and special attention should be given to ensure that (a) Sample collection and spectral generation should have minimal lag time (b) Case control samples should not cluster into distinct cluster times (c) archival samples are not used unless exact storage and handling information is available (d) the number of freeze thaw are kept to a minimum and (e) samples are stored at −80°C or lower to reduce ex vivo enzymatic activity of the coagulome.

199: Homology modelling of fumerate hydratase (FH) mutation protein and docking of fumerate hydratase (FH) mutation protein with 17-ketosteroid

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The following work is a Bioinformatics (proteomics) work developed by Computational tools. The drug designing procedure used here is Computer Aided Drug Designing (CADD). Uterine leiomyomas, commonly known as fibroids, are well-circumscribed, non-cancerous tumors arising from the myometrium (smooth muscle layer) of the uterus. In addition to smooth muscle, leiomyomas are also composed of extracellular matrix. The average affected uterus has six to seven fibroids. The protein Fumerate Hydratase (FH) mutation was found as one of the causes for the fibroids. Women with Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) have more uterine fibroids and onset at a younger age than women in the general population. Most women experience irregular or heavy menstruation and pelvic pain. Women with HLRCC and uterine fibroids undergo hysterectomy or myomectomy for symptomatic uterine fibroids at a younger age (less than 30 years) than the general population (45 years). Fumarate hydratase precursor (mutation) protein of Homo sapiens with ACCESSION NP_000134 (from NCBI database) was taken for our work. Homology modeling studies were done using 1JSW.pdb as template from Brookhaven (RCSB) Protein Data Bank and 3D structure of FH protein was modelled. Ashoka (Saraca asoca) is a small evergreen tree with large heads of orange-red flowers which are hightly perfumed at night. Traditional system of Indian Medicine shows Ashoka (Saraca asoca) to be effective against cases like Uterine fibroids, Dysmenorrhea, Hemorrhoids and Leucorrhoea. The bark, which is the drug, is reported to have a stimulating effect on the endometrium and ovarian tissue and is useful in menorrhagia due to uterine fibroids, in leucorrhea and in internal bleeding. It is useful in

all cases of uterine bleeding where ergot is indicated. The bark contains a steroid component (an estrogenic compound) ketosteroid and a calcium salt. It also has a stimulatory effect on the ovarian tissue and may produce an estrogen-like effect that enhances the repair of the endometrium and stops bleeding. The structure of 17-ketosteroid was made using the software ACD/ChemSketch and saved as MDL*mol file (17-ketosteroid.mol). Again, 17-ketosteroid.mol was opened with Argus Lab and saved as 17-ketosteroid.pdb which now acts as the ligand (drug) for the receptor FH protein. The receptor FH protein and the ligand 17-ketosteroid.pdb (drug) was docked by submitting the receptor and the ligand to HEX Server.

200: Aberrant glycosylation of plasma and synovial fluid proteins in rheumatoid arthritis

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Altered glycosylation of plasma proteins has been directly implicated in pathogenesis of rheumatoid arthritis (RA). To search for disease specific glycoproteins in plasma and synovial fluid, lectins of different carbohydrate specificity were used as tools. Glycoproteins with different sugar profile were isolated using lectin affinity chromatography and separated by 2D-gel electrophoresis. Glycosylation patterns of proteins from RA patients and healthy individuals were compared. Differential expression as well as glycosylation in number of proteins in the patients have been identified using various dyes. Identified proteins were analysed by MALDI-TOF/TOF. Sugar profiling were carried out using HPAEC-PAD. Aberrant glycosylation of cell surface glycoconjugates and plasma glycoproteins are implicated in various pathological conditions including RA. Results obtained from our study may provide useful clue in identifying diagnostic or prognostic markers and insight in the progression and pathogenesis in RA.

201: Quantitative proteomic approaches to identify biomarkers for hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and is frequently associated with cirrhosis, chronic hepatitis B (HBV) and C (HCV) virus infection. The objective of our study was to identify altered protein expression or glycosylation for use as biomarkers in HCC. Quantitation of proteins in complex biological samples using mass spectrometry has become feasible in recent years using methods that rely on incorporation of stable isotopes into peptides. Our approach was to use multiple technologies including the multiplexed isotope labeling for relative quantitation of HCC associated proteins using iTRAQ method and [18]O based quantitation and identification of tumor specific glycosylated proteins for the discovery phase and Western blotting and immunohistochemical labeling of HCC tissues for the validation phase. Two dimensional LC-MS/MS analysis and glycoprotein enrichment strategy generated more than 50,000 MS/MS spectra from which we were able to identify and quantitate more than 1,000 liver proteins in hepatocellular carcinoma. Over 200 proteins were found to be upregulated in HCC, which included both previously described markers as well as novel ones. Novel overexpressed proteins in HCC included fibroleukin, myeloid-associated differentiation marker, prothymosin alpha, high mobility group AT-hook 1 isoform a and leucine rich repeat containing 59. Over 100 proteins were found to be downregulated in HCC relative to adjacent normal tissues which included urea cycle enzymes, class I and class II alcohol dehydrogenases, fatty acid binding protein 1 and prostatic binding proteins. Western blotting confirmed the results of both upregulated and downregulated proteins. Using immunohistochemical labeling, we were able to validate overexpression of fibroleukin, myeloid-associated differentiation marker and fetuin in HCC and downregulation of urea cycle enzymes, fatty acid binding protein and prostatic binding proteins in HCC using tissue microarrays (n = 60). Lectin affinity enrichment was found to be advantageous to quantitate several interesting proteins, which were not detected in the whole proteome screening approach. Using lectin affinity followed by PNGase F digestion coupled to [18]O labeling, we identified 34 glycosylation sites. This study indicates that quantitative proteomic profiling of tumor tissue versus non-cancerous tissue is a promising approach for the identification of potential biomarkers for HCC.

202: A proteomic-based approach for the identification of potential Th-1 stimulatory novel proteins in a subunit vaccine (68–97.4 kDa) of soluble antigens of *Leishmania donovani* promastigotes that protects against fatal visceral leishmaniasis

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Visceral leishmaniasis (VL) caused by *Leishmania donovani* is a major parasitic disease prevalent in endemic regions of Bihar in India. In the absence of good chemotherapeutic options, there is a need to develop an effective vaccine against VL which should be dependent on the generation of a T helper type 1 (Th1) immune response. We have identified a fraction (F2 fraction) of soluble proteins from promastigote of new clinical isolate of *L. donovani* (2001) ranging from 68 to 97.4 kDa, which induced Th1 responses in the peripheral blood mononuclear cells of cured Leishmania patients and hamsters and also showed significant prophylactic potential. This fraction was further characterized by 2D-gel electrophoresis and MALDI-TOF-MS/MS and 33 proteins were detected. The F2 fraction was further sub-fractionated into seven sub fractions (F2.1–F2.7) according to the molecular weights by using Prep-Cell—a continuous elution SDS–PAGE and subjected to re-evaluation for their ability to induce cellular responses. Out of these, F2.4–F2.7 sub-fraction belonging to 97.1 to 89.9 kDa stimulated remarkable lymphoproliferative and IFN-γ, IL-12 responses in cured VL patients and in endemic controls but IL-10 was not significantly detected. Similarly, significant lymphoproliferative responses and nitric oxide production were also noticed

in cured Leishmania infected animals indicating an element of uniformity in responses between hamster and human. But when we pooled F2.4 to F2.7 together it shows maximum cellular responses as compare to individual sub fractions. The immunostimulatory sub fractions were further characterized by one-dimensional electrophoresis followed by MALDI-TOF-MS. A total of 18 proteins were identified including five hypothetical/unknown proteins. Major immunostimulatory proteins were identified as Elongation factor-2, p45, Heat shock protein (HSP) 70, HSP 83, Aldolase, Enolase, Triosephosphate isomerase, Protein Disulfideisomerase and Calreticulin.

203: Identification of cancer markers and their correlation to cancer diagnosis and prognosis

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A tumor marker is a biochemical indicator for the presence of a tumor. Clinically, it is a molecule (isoenzyme or glycoprotein) that can be detected in plasma or other body fluids under normal conditions, but their concentrations become abnormally high in presence of tumorous growth. There are many different tumor markers, each indicative of a particular disease process. In oncology, they are used to help detect the presence of cancer. Some of the typical cancer markers with which we worked were: Alpha-fetoprotein or AFP (liver cancer), Carcinoembryonic antigen or CEA (colorectal cancer), Human chorionic gonadotropin or HCG (testis cancer), CA 19-3 (breast cancer), CA 125 (ovarian cancer), Prostate specific antigen or PSA (prostate cancer). Cancer markers are mainly being used for the following four purposes: (1) screening a healthy population or a high risk population for the presence of cancer; (2) making a diagnosis of cancer or of a specific type of cancer; (3) determining the prognosis in a patient; (4) monitoring the course in a patient in remission or while receiving surgery, radiation, or chemotherapy. We analysed the level of different cancer markers in healthy and diseased conditions within the body and correlated them with the diagnosis of that specific type and stage of cancer. In addition, we also provided an insight into the technique of immunodetection of cancer markers.

204: Quantification of inflammatory mediators in diabetic nephropathy: an experimental design approach

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Background: The complex pathophysiology of diabetes has sparked the development of novel proteomic techniques that require proper design and validation. This study focuses on multiplexed analysis of cytokines in diabetic nephropathy.
Methods: Multiplexed enzyme linked immunosorbent assay ELISA, Gel electrophoresis followed by mass spectrometry were performed on plasma from 30 diabetic nephropathy patients. C-reactive proteins CRP, Interlukin-6 IL-6, Interlukin-10 IL-10, tumor necrosis factor-a TNF-a, myeloperoxidase were measured with ELISA. Experimental design methodology applied to perform gel electrophoresis and LC-MS/MS analysis of cytokines. Detection limits for between and within runs were determined. Experimental design methodology was employed to conduct method robustness and intermediate precision. Results: Correlation between the multiplexed assays of ELISA was good for CRP, IL-6, IL-10, TNF-a and myeloperoxidase. Within and between run impression values for the multiplex method were <15%.
Conclusion: The application of different mathematical tools is therefore a prerequisite for the realization of the robust results. Possible restrictions when it comes to choosing the setting of a specific parameter will be discussed. A stepwise optimization strategy using an experimental design is proposed, that hopefully will aid scientists to optimize the performance of such an experimental design approach for biomarker development and validation.

205: Proteomic analysis of differentially expressed proteins in swiss mice following glyphosate exposure

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Gene expression analysis has been widely used in predicting the course of pesticide induced carcinogenicity. However, very few reports are available regarding differential protein expression pattern. Recent advances in proteomic analysis allow proteins of interest to be identified by their expression and/or modification pattern in 2-dimensional gel electrophoresis (2-DE) analysis rather than using the traditional approach of translating gene expression data. Using 2-DE, we compared the differential expression pattern of proteins, modulated in response to exposure of a wide spectrum organophosporous herbicide, glyphosate and known carcinogens namely 7,12-dimethylbenz[a] anthracene (DMBA), benzo[a]pyrene (B[a]P) and 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) on skin of Swiss albino mice. Image matching derived from four sets showed total of 158 spots. Out of 158, 8 protein spots and a cluster of spots in an area were common in between TPA and glyphosate exposed skin tissues whereas only 3 and 4 spots were observed similar in DMBA and B[a]P as compared to glyphosate treated skin, respectively. The data suggests that glyphosate regulates similar number of protein spots as that of TPA which is a known tumor promoter. Besides this, no protein spots were shown to be significantly altered in glyphosate exposed as compared to unexposed skin tissue. Therefore, our results suggest an improved prediction of toxicity/tumorigenicity of pesticides by means of protein markers using toxicoproteomics approaches, which could in the future lead to a better understanding of pesticide carcinogenesis at an operational level.

206: Changes in expression of CD80, CD86, TLR2 and TLR4 in CD14-mononuclear cells after vaccination with an oral cholera vaccine

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Introduction: Toll-like receptors (TLRs) are a family of pattern-recognition receptors, representing an important link between innate and adaptive immunity. Cell-surface TLRs (e.g., TLR2 and TLR4) initiate innate immune responses to microorganisms' leading to upregulation of co-stimulatory molecules (e.g., CD80 and 86), marking the transition to the adaptive pathway. Dysregulation of these proteins (TLR2, TLR4, CD80 and CD86) may result in a poor response to vaccines against intestinal bacteria.

Aim: To test whether levels of TLR2, TLR4, CD80 and CD86, increase after vaccination against Vibrio cholerae.

Methodology: As a part of a large study on immune responses to an oral cholera vaccine (composition: lipopolysaccharides of killed strains O1 Inaba Cairo 48 and El Tor Phil 6973; O1 Ogawa Cairo 50 and O139 4260B) in a slum area of Kolkata (India), peripheral blood from a subset of unrelated individuals (n = 130) was collected prior to vaccination (Day 0) and 3-days post-vaccination (Day 3) and levels of TLR2, TLR4, CD80 and CD86 were estimated by flow cytometry. Vaccines with no detectable levels of these proteins (22–38%) either pre- or post-vaccination were excluded from analyses. Post-vaccination, the fold-increase in expression (Day-3 vs. Day-0) of each protein was statistically analyzed using ANOVA.

Results: (1) Age or gender had no significant effect on the fold-increase for any of these proteins. (2) Vaccines with post-vaccination increase in protein expression, the mean fold-increase was significantly >1.0 for CD80 (mean = 1.35; p = 0.019) and marginally significant for TLR4 (mean = 1.46; p = 0.056). (3) 17% of vaccines showed >2-fold increase for CD80 and TLR4. (4) Those with an increase in post-vaccination levels (43.13% for CD80; 53.84% for CD86; 43.84% for TLR2; 39.51% for TLR4), the mean fold-increase was 2.4 and 2.9 for CD80 and TLR4, respectively, while those for CD86 and TLR2 remained unchanged.

Conclusions: (1) 40–54% of vaccinees showed an increase in post-vaccination levels of one of the four proteins. (2) Among them, the mean fold-increase was ~2.5 for CD80 and TLR4, but not much higher than 1.0 for CD86 and TLR2. (3) A small proportion of 6.3% of the vaccinees have >2-fold increase in the levels of TLR4 and CD80 expression post-vaccination.

207: Two natural missense mutations at intersubunit contact domain in human pyruvate kinase M2 (PK-M2) isozyme with differential behavior

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Two independent mutations in an enzyme, PK-M2, replacing Lys 422 by Arg (K422R) and His 391 by Tyr (H391Y) in the same domain, altered both the structure and the function of the enzyme differentially. These natural mutations were reported earlier independently from a Bloom syndrome cell line and Bloom syndrome patient, respectively. The wild type PK-M2 and H391Y, K422R mutants, despite retaining their homotetramer state, showed completely differential effect on enzymatic activity. Where the H391Y mutation resulted in loss of allosteric nature of protein by increasing its substrate affinity by 6 times, the K422R showed reduced substrate affinity. H391Y showed only 20% loss of total activity which was compensated by the binding of heterotrophic activator FBP, which unexpectedly had nearly no effect on its affinity for substrate (PEP) while K422R exhibited nearly 80% loss in activity with high increase in affinity even in comparison to wild type PK-M2. Apart from losing co-operativity, the H391Y mutant also attained structural rigidity, showing thermal stability, stability over change in pH and resistance to the effect of allosteric inhibitor phenylalanine, while K422R behaved as sensitive for temperature, and allosteric inhibitor as that of wild type PK-M2. Both the mutants showed a slight shift of optimum pH from 7.4 to 7.0. The differential impact on tertiary structure of H391Y and K422R was revealed by intrinsic Trp fluorescence and CD spectroscopy. It is proposed that PK-M2 could be a target for down regulation of its activity either through mutations as reported here or other alternative mechanisms to provide a cell an advantage to grow uncontrollably, as has been reported recently (Christofk et al., Nature, 2008). This would also explain the proneness to cancer especially in the genetic background provided by Bloom syndrome that is susceptible to a variety of cancers in early age.

208: A multi-pronged proteomic approach to study pancreatic cancers

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Pancreatic cancer is one of the most lethal of all the solid malignancies. This is largely due to late clinical presentation of disease and lack of effective therapy. Because of this, pancreatic cancer has one of the lowest 5 year survival rates among all solid malignancies, emphasizing the critical importance of developing new biomarkers for early detection and identifying novel targets for therapeutic intervention. All of the currently available biomarkers lack the desired specificity and sensitivity. With the recent advances in mass spectrometry, it is now possible to analyze proteome at a global scale. Also, the availability of various quantitative proteomic methods allows researchers to compare multiple disease samples simultaneously thereby facilitating the identification of differentially regulated proteins. In order to carry out a systematic study to identify potential biomarkers of pancreatic cancers, we have employed a multi-pronged proteomic approach using stable isotope labeling with amino acids in cell culture (SILAC) and isobaric tag for relative and absolute quantitation (iTRAQ) based quantitative proteomics. SILAC was used to carry out phosphoproteomics and membrane proteomics studies using pancreatic cancer cell lines while iTRAQ was used in the analysis of surgically resected pancreatic cancer tissue specimens. As a more direct approach to identify potential pancreatic cancer biomarkers from body fluids, serum proteomics and urinary proteomics were also carried out. Finally, in order to integrate all the data scattered across the published literature, we have developed a compendium of candidate biomarkers of pancreatic cancer published till date. Many of the potential biomarkers identified from the above approaches were novel and have been validated using immunohistochemical labeling and ELISA based methods.

209: Cyclooxygenase 2-dependent multidrug resistance 1 overexpression confers resistance to imatinib in chronic myeloid leukemia cell line-K562

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Resistance to the Ableson protein tyrosine (Abl) kinase inhibitor, imatinib mesylate, has become a critical issue for patients in advanced phases of chronic myelogenous leukemia. In the present study, we have generated imatinib-resistant chronic myeloid leukemia cell line (IR-K562) and studied the possible mechanisms of resistance development. Preliminary studies indicated that Bcr-Abl over expression and kinase domain mutations are not involved in the resistance development in IR-K562 cells but revealed the over expression of COX-2 and MDR1 in IR-K562 cells suggesting the possible involvement of COX-2 in the development of resistance to imatinib via the regulation of MDR1 expression. Furthermore, down regulation of MDR1 upon COX-2 knockdown by siRNA or COX-2 inhibition by celecoxib, a selective COX-2 inhibitor, further supported such a possibility. The study showed a decrease in the PKC levels by COX-2 knockdown/inhibition in IR-K562 cells and activation of PKC by addition of PGE2 to K562 cells, suggesting a role for PKC in the COX-2-mediated induction of MDR1. Further, studies on the mechanism of induction of COX-2 and MDR1 by imatinib in IR-K562 cells suggested a role of ROS in the activation of NF-kB and AP1 transcription factors that regulate COX-2 expression. In view of the COX-2 role in resistance development, further studies were taken up to evaluate the efficacy of COX-2 selective inhibitor, celecoxib, on growth of IR-K562 cells. The results clearly indicated that celecoxib is more effective in IR-K562 cells with a lower IC50 value of 10 μM compared to an IC50 value of 40 μM in K562 cells. This increase in the sensitivity of IR-K562 cells towards celecoxib suggested that the development of resistance in IR-K562 cells is COX-2 dependent. Celecoxib-induced apoptosis of IR-K562 cells led to release of cytochrome c, PARP cleavage, decreased Bcl2/Bax ratio and pAkt. This was further supported by the results from in vivo studies, using xenograft mouse animal model, which showed a clear decrease in the tumor size and down regulation of COX-2, MDR1 and VEGF (angiogenic factor) in celecoxib-treated animals (20 mg/kg body weight) compared to imatinib treated mice (50 mg/kg body weight).

210: To study the effect of benzimidazole analogues (DNA minor groove binding ligands) on the protein expression of U87, a glioblastoma cell line under Ionizing radiation

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Ionizing radiation causes damage to human kind, plants and earth in several ways. Exposure to radiation whether accidentally or unavoidably may cause a lot of mutagenic and clastogenic effects in any living organism. Ionizing radiation deposits energy that injures or destroys cells by damaging the genetic material, resulting in the induction of apoptosis in cells that have accumulated DNA damage. Moreover, during radiotherapy, radiation damages both cancer and normal cells during the treatment of localized solid tumors. The present work focuses on the development of a series of analogues of benzimidazole (DNA minor groove binding ligands) as Radioprotectors, substances which protect the cells against radiation induced damage. Our aim is to elucidate the molecular mechanism of action of these benzimidazole analogues, which confer radioprotection and are non cytotoxic. As a logical follow up, Microarray hybridization and data analysis revealed a number of cell cycle genes to be variably regulated in response to ligand and radiation treatment.

Considering the fact that two dimensional gel electrophoresis with immobilized pH gradients (IPGs) combined with protein identification by mass spectrometry (MS) is currently the workhouse for proteomics, protein expression profiling was done. Hence, protein expression was observed by 2D PAGE electrophoresis using whole cell lysates (U87 cell line—control, ligand treated, radiation treated and both ligand and radiation treated). The protein pattern differences between different experimental samples were studied by analyzing images. For each treatment group, a number of protein spots were found to be differentially expressed which were subsequently subjected to trypsin digestion and analyzed by Mass Spectrometry. Out of the several protein spots identified by MS/MS Sequencing, a few functionally relevant proteins—Nucleophosmin, Poly rC binding protein 1 and Heat shock cognate protein were identified.

211: Use of in-silico approaches to identify promoter elements and transcription factors associated with malignancy in ovarian cancer followed by validation of potential targets in tissues and serum samples of patients with ovarian cancer using proteomic approaches

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Aims: Epithelial ovarian cancer (EOC) presents in the later stages of the disease. Regulatory genes/transcription factors (TFs) control the functions of various genes associated with cancer development. We hypothesized that over expressed TFs associated with EOC pathogenesis could be useful tissue and serum markers for malignancy associated with EOC.

Methods: Using microarray data we shortlisted candidate genes associated with apoptosis and cell structure integrity. Using promoter and TF binding analysis we shortlisted target TFs that could play a key role in malignancy associated with EOC. Using immunoblotting and IHC we validated the in silico predictions and associated these markers with CA125 for their clinical utility.

Results: Using a combined genomic-bioinformatics and proteomic approach we identified potential regulatory genes associated with the pathogenenicity of EOC. EOC-specific microarray data were examined for over-expressed genes. Promoter models of over-expressed genes were determined and bioinformatic analysis implicated transcription factors ZEB1, E2F5, PAX8 and ELF3, involved in cell proliferation, as promising regulatory targets in early stage disease. Additional analysis found E2F5 as a prime candidate for further

study. Our in silico observation was supported by tissue microarray experiments that showed E2F5 expression only in EOC samples and not in normal and benign tissues as observed in a total of 135 (111 tumours and 24 normal) tissue array (45.6% of EOC tissue samples) and 144 serum (Normal: 56, benign: 40 and malignant: 48) 81.25%, p = 0.0001 patients with EOC. Analysis of clinical characteristics of this protein used in different combinations with CA125 for distinguishing malignant cyst from benign cyst shows increased sensitivity (97.9%; increase from 87.5% if CA125 only is used) and more importantly increased specificity (72.5%; increase from 55% if CA125 only is used). This significantly improved accuracy suggests improved diagnostics of EOC. Overall, our findings provide evidence that some of the cell-cycle regulatory proteins might play a significant role in EOC pathogenesis. Conclusion: Our findings show involvement of E2F genes in EOC development and progression. Larger prospective studies are essential to validate our findings. We have demonstrated the application of microarray analysis could facilitate the identification of genes, genetic pathways, and proteins involved in the pathogenesis of EOC and their utility as potential serum markers.

212: BIOBASE Knowledge Library™ (BKL) disease view and HGMD®: a comprehensive literature-derived disease information management system

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The **Biobase Knowledge Library™ Disease View** presents detailed information on 1988 different human diseases with the aim of helping researchers to understand the molecular mechanisms underlying human disease. More than 5,234 proteins are associated with disease terms resulting about 30,000 protein-disease links. Disease View provides manually curated links between genes and MeSH terms in four major categories: biomarker, therapeutic target, molecular mechanism and negative correlation; as well as information on expression of gene or protein in a particular disease condition and knockout models. Each disease report provides detailed information about the effects of molecular alterations on the biological processes associated with the respective gene. Information is provided for 4,284 biomarkers, 1,311 therapeutic targets, and 94,461 annotations comprising 4,937 polymorphisms, 12,749 mutations, etc. Disease View is closely integrated with TRANSFAC®, a database on gene regulation and transcription factors, and TRANSPATH®, a database on signal transduction and metabolic pathways. Integrated databasing allows the user to make sophisticated queries and to generate new information based upon multiple facts brought together from the dispersed literature. As an example workflow through the integrated BKL™, one could identify all proteins associated with metabolic disorders, as well as those involved in signaling and metabolic pathways and gene regulatory networks. With the help of the accompanying tools, one could (i) retrieve the promoters of the corresponding genes, (ii) identify putative TF binding sites and (iii) analyze networks to find potential key nodes. An exclusive feature of

the BKL™ is the interlinking with the internationally acclaimed **Human Gene Mutation Database (HGMD®)** curated at Cardiff University. The HGMD® represents a comprehensive core collection of germ-line mutations in nuclear genes underlying or associated with human inherited disease. The database contains over 79,000 different lesions detected in 3,000 different human genes, with new entries currently accumulating at a rate exceeding 9,000 per annum. HGMD® records the first report of a disease-causing mutation or disease-associated/functional polymorphism. The data comprise single base-pair substitutions in coding, regulatory and splicing-relevant regions of human nuclear genes, micro-deletions and micro-insertions, indels, repeat expansions, gross lesions and complex rearrangements.

213: Methylation status of EpCAM in y79 retinoblastoma cell line: proteomic analysis shows modification of various other proteins

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Retinoblastoma (RB) is the most common intraocular malignancy of infancy and childhood in which epithelial cell adhesive molecules (EpCAM) was found to be over expressed and associated with invasion of the choroids and optic nerve. But surprisingly Y79 RB cell line did not show any EpCAM expression. However the molecular mechanisms responsible for the downregulation of EpCAM in Y79 RB cell line was not known. DNA methylation is an important mechanism for inactivating various genes during tumourigenesis and progression. The presence of a CpG island in the TACSTD1 gene promoter and first exon, encoding EpCAM, led us to investigate in this study whether EpCAM expression can be influenced by DNA methylation. We examined the methylation status of the TACSTD1 promoter region RB cell lines using bisulfite sequencing. We found the promoter of EpCAM-negative Y79 RB cell line to be methylated to a higher degree. Demethylation of cell lines was performed using 5azacytidine (AZC). TaqMan gene expression assays were used to quantify the mRNA expression of EpCAM normalized against two endogenous controls using geNorm software. Immunoflurosence (IF) was carried out using EpCAM antibody. EpCAM RNA and protein expression could be partially restored by treating cells with AZC. By real-time PCR the EpCAM RNA expression increased more than 250-fold after 5 days of incubation with AZC compared to the untreated cell line, and by IF it was resulted in the expression of detectable amounts of EpCAM protein on the cell surface. We have also attempted to determine the changes in the other protein profile changes in AZC treated Y79 cell line. The strategy applied makes use of proteomics technologies to reveal and identify other proteins that are differentially regulated in control and in AZC treated cells. We have identified and analyzed 16 differentially expressed proteins in Y79 using 2DE-MS approach. The identity of most of these differentially expressed proteins was determined by 4800 MALDI TOF TOF. We found 13 proteins are up regulated and 3 proteins were downregulated in AZC treated cells compared to untreated Y79. The functional significance of all the proteins was identified from swissprot. Taken together, these data suggest that epigenetic inactivation of genes by DNA methylation can be reversed by treatment with the DNA methylation inhibitor AZC. Our study will provide a basis for further investigation into metabolic pathways affected by demethylating drugs and their mode of action.

214: Analysis of proteins and their fragments released by human glioma cell lines as potential tumor markers

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Astrocytoma is the most predominant tumor type among gliomas—the primary tumors of the central nervous system. They are highly heterogenous and malignant and contribute significantly to the cancer-related deaths. Location of gliomas and vague symptoms cause complexity in identifying the tumors in the early stages. We have observed that a major intermediate filament protein of astrocytes—the glial fibrillary acidic protein (GFAP) undergoes fragmentation in tumor cells and can cause cascade effects on differentiated cellular state. GFAP fragments may also be released into the exterior. Some of the GFAP peptides detected in the blood plasma of glioma patients are being characterized in this perspective and their quantification is being studied. During tumor proliferation and metastasis, tumor cells may also release other proteins such as membrane proteins or extra cellular matrix proteins, into the surrounding environment and may serve as potential tumor markers. To understand such plausible protein secretion, we are studying proteins that are secreted by cell lines developed from human gliomas into their culture media. On 1D and 2D gel electrophoresis of proteins from the conditioned media of glioma cell lines and their comparison with the cellular protein profiles, we observed number of proteins that were significantly enriched in the conditioned media. These proteins are being identified by Peptide mass finger prints or by LC MS/MS based peptide sequencing and may be additional useful candidates for examination in the plasma of glioma patients as potential markers for diagnosis and prognosis of gliomas.

215: Proteomics of the outer membrane of native and β-lactam resistant strains of *Acinetobacter baumannii*

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Acinetobacter baumannii is a gram negative opportunistic pathogen, responsible for nosocomial infections in hospitals worldwide. The bacterial strains of *A. baumannii* are widely prevalent in nature and are commensals of human skin, respiratory tract and genitourinary tract. In the recent past *A. baumannii* has developed high antibiotic resistance against β-lactams including the more recent, most effective carbapenems. We report for the first time, identification of outer membrane proteins of 50 clinical β-lactams resistant isolates and compared them with that of native ATCC 19606 strain using DIGE, Difference in fluorescence 2D gel electrophoresis and mass spectrometry. A total of 35 proteins of which 12 proteins have been identified as novel in ATCC and showed transport function. The expression of these 12 proteins is down-regulated in resistant strains of *A. baumannii*. Novel proteins were also identified in the resistant strains of *A. baumannii* as the RND-type efflux systems, CarO isoforms, OmpW isoforms and several potential virulence factors. This proteomic study will provide a platform to understand the complex nature of β-lactam resistance in *Acinetobacter baumannii*.

216: Embryonic stem cells, their biomedical potential and proteomics approach for quality control

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Stem cells have great potential for developing new approaches for the treatment of degenerative diseases including neurons, skeletal and cardiac muscles, beta cells of pancreas, and cancer cells. Cell replacement therapy with stem cell and their derivatives are thus an important biomedical tool. A better understanding of the molecular pathways, regulatory networks and their dynamics, which determine their diverse differentiation fates, is needed for these therapeutic approaches to be successful. With these objectives, we have been studying protein expression in mouse embryonic stem cell lines (R1-9 and ABI), as model system. We have extensively studied their protein profiles by ESI LC MS/MS approach, integrated the data with transcriptomics studies as well as with proteomics studies from other laboratories. We have thus identified more than 2,000 proteins expressed in stem cells with high confidence. Pathway analysis of these proteins was carried out using KEGG, IPA, GenMAPP and their gene ontology classification revealed transcription regulators, signal transducers, cell cycle and differentiation molecules along with other general classes of proteins. Based on the specific proteins expressed, putative regulatory pathways operational in stem cells could be constructed. Such information on protein expression and plausible biochemical pathways and networks operational in the stem cells and their derivatives would be useful in defining cell states and developing quality control methods for their biomedical application.

217: Mechanistic studies of antiproliferative activity of *Phyllanthus emblica* extract on HeLa cells

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Introduction: The extracts from *Phyllanthus emblica* tree (amla) have been shown to possess several pharmacological actions including anti-carcinogenetic and anti-tumor activities. The present study was conducted to analyze the effect of *P. emblica* on cancer cell lines and the molecular mechanisms by which the compounds present in the extract are responsible for the antiproliferative effect and morphological changes.
Method: The antiproliferative effect of *P. emblica* extracts was assessed on different cancer cell lines like HeLa (Cervical carcinoma), HepG2 (liver cancer) etc. Cell viability was checked with MTT assay and microscopic studies were carried out simultaneously to evaluate morphological changes. Flowcytometry studies of cells treated with *P. emblica* extract were carried outto evaluate its effect on cell cycle. Expression of Ki-67, an actively proliferating cell nuclear protein, and Cyclin D1, a G1-S cell cycle checkpointprotein,

were checked by immunoblotting. To check the role of apoptosis in antiproliferative activity of *P. emblica* extract, caspase3 activity was evaluated using DEVD-AFC assay. The proteome profile was studied to detect and identify proteins whose expression is altered with extract treatment in cervical carcinoma HeLa cell line, in order to unravel the mechanisms underlying the antiproliferative effect of emblica extract. Results: The results indicated that *P. emblica* extract showed antiproliferative and inhibitory effects on cancer cells. The treatment of cells with extract arrested the cells in G1 phase of cell cycle as indicated by the flowcytometry. Western blot analysis of Cyclin D1 showed gradual decrease in expression with increase in concentration and expression loss of Ki-67 with extract treatment. Increase in emission of fluorescence in DEVD-AFC caspase activity assay was observed, which is directly proportional to the activity of caspase3. Differential proteome analysis of cell lysates of treated and untreated control cells showed 20 differentially expressed spots which were identified by nano LC MS analysis. Conclusion: The changes in morphology, cell cycle arrest in G1 phase, changes in expression of Ki-67 and Cyclin D1 proteins and increase in caspase activity showed that the antiproliferative effects were due to arrest of cells in G1 phase thereby leading to apoptosis. The proteins identified through proteome analysis also supported the induction of apoptosis. Both caspase mediated and caspase independent pathways of apoptosis were induced.

218: Identification of differences in protein pattern and differentially expressed protein in wildtype *Schizosaccharomyces pombe* and Pol-gamma null strain depleted of mitochondrial genome using two dimensional gel electrophoresis and mass spectrometry

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Two dimensional gel electrophoresis coupled with mass spectrometry is an excellent approach in proteomic research to identify protein expression pattern on a global scale as well as post translational modification and classify proteins based on their abundance and biochemical functions. Though the technique is sensitive, key questions remain to characterize protein on a whole cell proteome-wide scale. To improve the potential of 2D gel separation we systematically focused the protein samples in the pH 3–10, pH 4–7 and pH 6–9 linear gradient gels to compare the proteomic pattern of fission yeast *S. pombe* and Pol-gamma null mutant in the second dimension electrophoresis and to identify the differentially expressed proteins using LS-MS/MS. The proteomic analysis of wildtype MBY192 strain shows a specific pattern of protein movement based on the pI and MW. Comparing wildtype and pog1-delta data showed dramatic changes in the energy, amino acids and glucose metabolism in the mutant cells. The list of enzymes and proteins that catalyze reactions in citric acid cycle, glycolysis and oxidative phosphorylation were either detected in low amounts or completely absent in the mutant phenotype. The major proteins involved in the citric acid cycle viz. isocitrate dehydrogenase, malate dehydrogenase, dihydrolipoamide succinyl transferase were not detected in the mutant indicative of deregulated mitochondrial biosynthetic pathway and indirectly affect DNA replication and elongation. The identification of significantly altered proteins in the selected region provides an annotated pog1-delta mtDNA mutant related proteome that can be used as a template

against other stress factors induced or mutation induced changes. We established and report *S. pombe* reference proteome map using coomassie blue stain and the simplicity of staining procedure allows qualitative identification of differentially expressed and post-translationally modified proteins between groups.

219: Functional characterization of putative disease proteins in Type 2 Diabetes Mellitus

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Type 2 Diabetes Mellitus (T2DM) is a polygenic disorder with both gene-environment and protein–protein interactions influencing the disease risk. A huge effort to unravel the risk factors has been undertaken worldwide for over a decade but with little success. On these lines, we designed a system biology approach to identify plausible disease candidates by assigning a score called weight value (Wv) ranging from 0 to 1 based on domain interactions and subsequent network analysis. Using this approach we identified certain already known T2DM proteins and also captured proteins of unknown functions and several new candidates for this dreaded disease. Our analysis revealed Lipin family member: Lipin-2 and Lipin-3 to be probable disease candidates in T2DM. Hence, to validate our approach, the functional characterization and identification of Lipin family was done in zebrafish model system. There are three members of lipin family having Phosphatidic acid phosphate (PAP) enzyme activity, responsible for generating diacylglycerol utilized in the synthesis of triacylglycerol (TAG). The regulation of TAG storage is important in human disease because both excessive and inadequate food storage might lead to abnormalities like insulin resistance and diabetes. Protein sequence alignment and phylogenetic analysis of three unknown proteins in zebrafish showed high similarity to that of three human lipin family members. Expression analysis using Reverse transcriptase PCR of total RNA obtained from different tissues of zebrafish revealed the expression of Lipin-1, 2 and 3 proteins in intestine, gills, muscle, ovary, liver and kidney. In situ hybridization of Lipin members (Lipin-1, 2 and 3) revealed that all the three had expression pattern centered in eye and head region of 24 h post fertilization (hpf), 36hpf, 48hpf and 72hpf embryos. Thus, further studies assessing the roles of these three proteins in zebrafish would assist in defining their explicit role in human diseases.

220: Development of novel biomarkers for breast cancer in north Indian population

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Breast cancer is one of the most common malignancies among women worldwide. Despite tremendous advances in screening, diagnosis, and treatment, the causes of this disease remain elusive and complex. Protein-based breast cancer biomarkers are a primary resource for breast cancer detection at the earliest and most treatable stages of the disease. Blood is an easily accessible source of proteins, which have diagnostic value, as it is in contact with practically all

tissues in the human body. The vast dynamic range of protein abundance in blood plasma represent a major challenge in applying a proteomic based strategy for their identification. From an experimental design point of view, most cancer biomarker studies, including those aimed at identifying markers for early detection, are initiated with analysis of specimens from newly diagnosed subjects. The discovered candidate biomarkers are subsequently investigated for their utility for early cancer diagnosis. Differential protein expression in the breast cancer and healthy individual's plasma samples was investigated by two dimensional gel electrophoresis (2-DE), and spots showing a significantly expression between the two were analyzed. Image matching derived from two sets showed total of 112 spots. Out of 112, 2 proteins were up-regulated or present in patient's plasma samples as compared to control and 3 protein spots were found to be differentially expressed by means of quantitative analysis. This investigation can lead to the development of potential biomarker that may have clinical utility in discovering biomarkers of breast cancer.

221: Pigment epithelium-derived factor treatment for cervical cancer

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The aim of the study is to find the role of Pigment epithelium-derived factor (PEDF) in the treatment of cervical cancer. PEDF with its antitumorigenic activity is an inhibitor of angiogenesis that is involved not only in protection against inappropriate vessel growth in the adult but also seemingly in normal embryonic development as well-where avascularity is important as in cornea, vitreous, and within the inter-photoreceptor matrix. Bioinformatics tools and databases like NCBI, BLAST, CLUSTALW, TEXSHADE, PRODOM, CATH, SIGNALP, PFAM SWISS-PROT and others are used for the study. PEDF has been identified in the protein expression patterns of squamous cell carcinoma. We found a conserved pattern of amino acids which can be responsible for its anti tumour activities. Examination of PEDF suggests that its increased expression contributes to tumour suppressions. Thus it may serve as a multifunctional antitumor agent suggesting that its clinical administration could stimulate a multi-faceted antitumor feedback loop with the potential to limit and possibly regress tumour growth in cervical cancer.

222: Glycosylated and thermostable proteins in serum of type 2 diabetes mellitus patients

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In this study, two protein fractionation approaches for the analysis of type 2 diabetes mellitus (T2DM) serum proteome have been applied: (i) affinity chromatography by using lectins to capture and separation of glycoproteins; (ii) heat treatment to remove thermostable proteins. The protein fractions were then separated by two-dimensional gel electrophoresis (2-DE). The spots were further excised, trypsin-digested and analyzed by nanoLC-MS/MS. Comparative analysis between T2DM patients and healthy individuals allowed us to identify a number of glycosylated proteins that were differently expressed in the patients. Of those proteins, haptoglobin alpha1, haptoglobin alpha2, haptoglobin beta, complement C3, Zinc alpha2 glycoprotein were highly up-regulated, while Ig J chain was down-regulated in T2DM patients. The result of protein ID also showed that apolipoprotein A-I, transthyretin, haptoglobin alpha 2, haptoglobin alpha 1 were thermostable and up-regulated in T2DM serum. It has suggested that the analyzed proteins could be a significant sign to access T2DM and need to be further characterized.

223: Glutathione-S-transferase with reduced allergenicity has therapeutic potential in mice model of asthma

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Oxidative stress contributes in the pathogenesis of asthma. Glutathione-s-transferase (GST) is an antioxidant and its deficiency increases the risk of developing asthma. The present study investigates the effect of GST and mutated GST (mGST) with reduced IgE binding in murine model of airway inflammation. BALB/c mice were immunized i.p. with ovalbumin (10 µg/100 µl PBS) on days 1 and 14 and challenged i.n. on days 28, 29, and 30. Mice were administered intranasally with GST, mGST, lipoic acid (LA) and PBS after 1 h of each OVA challenge. Mice were sacrificed on day 31 and lungs were used for histology. Total and differential cell count, IL-4, IFN-γ and oxidative stress were measured in BALF. Immunoglobulins were determined in sera. GST and mGST have similar enzymatic activity but mGST had reduced IgE binding (44% reduction). mGST treated mice showed significantly reduced total cell count and eosinophils in BALF as compared to GST administered groups (p < 0.01). Lung inflammation score in terms of eosinophilic infiltration and mucous secretion was lowest for LA treated group followed by mGST and GST administered groups. IL-4 was significantly reduced for mGST group compared to GST group mice (p < 0.01). However, no change was observed in IgG subtypes. Oxidative stress in BALF of both mGST as well as GST administered mice were reduced significantly in comparision to PBS instilled group (p < 0.01). These data suggests that mGST with reduced IgE binding has potential to limit airway inflammation in bronchial asthma.

224: Identification of Rheumatoid Arthritis causing protein and interpreting the treatment for the disease

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Rheumatoid Arthritis is an autoimmune disease that occurs when the body's own immune system mistakenly attacks the synovium. It is mainly characterized by inflammation of the lining or synovium of the joints and it can lead to long-term joints damage, resulting in chronic pain, loss of function and disability. Homo sapiens major histocompatibility complex, class II, DR beta 4 (HLA-DRB4) HGNC: 4952 [NM_021983 (from NCBI database)] was taken for our work. The structure of HLA-DRB4 protein was modelled using homology modeling. Because rheumatoid arthritis presents itself on many different fronts and in many different ways, treatment must be tailored to the individual, taking into account the severity of arthritis, other medical conditions and individual lifestyle. Current treatment

methods focus on relieving pain, reducing inflammation, stopping or slowing joint damage and improving functioning and sense of well-being. Ayurvedic herbs have been used since thousands of years to produce herbal remedies. Nowadays it is a well established fact that herbal remedies are more suitable to human body than isolated chemical medicines. There are a number of herbs that work synergistically to reduce chronic joint to the Burseraceae family, including *Boswellia serrata*, tree is commonly found in India. The therapeutic value of its gum (guggulu) has been known. It possesses good anti-inflammatory, anti-arthritic and analgesic activity. The goal of this study was to evaluate the effectiveness of boswellia extract (boswellic acid) in the treatment of rheumatoid arthritis with minimal side effects. The structure of boswellic acid (obtained by CHEM3D) was converted to boswellia.pdb was successfully docked to HLA-DRB4 protein using HEX software.

225: Rapid identification of human plasma proteins by mass fingerprinting using PLASMASS search engine

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Protein identification by Peptide Mass Fingerprinting (PMF) has become an integral and essential step of clinical and biochemical studies. Protein fragments' masses (from in-gel digestion by enzymes) are ascertained by sensitive Mass Spectrometers (MALDI, ESI) and are searched against a database containing theoretically cleaved (in silico digested) masses from the available proteins. The multitude of PMF databases available, do not specifically address Human Plasma Proteins. We have developed PLASMASS, a database for rapid and accurate identification of human plasma proteins. PLASMASS is based on in silico fragmentation, wherein all possible combinations of fragments of available human plasma proteins are generated to make the database exhaustive and sensitive. We provide additional flexibility by letting the user add more proteins 'on the fly' to the database repository, after which the mass spectrometry data can be searched against this increased set of proteins. This tool, though currently focuses on Human Plasma Proteins, can easily be extended to create customized database for any functional class of proteins from any taxa.