Recognizing the Proteomic Patterns of Induced Toxicity with 1D-ZOOMER Approach

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Response of cell proteome on the administration of chemical entities is important for evaluating the drug candidates and while designing the personalized drug courses. Here we investigate the changes in the proteome of additionally purified microsomal fraction of hepatocytes resulting due to the treatment of experimental animals with Phenobarbital (PB) and 3-Methylcholanthrene (3-MC). These compounds are known to invoke different mechanisms of molecular toxicity.

Many of toxicity defense proteins are buried into the membrane of endoplasmic reticulum, therefore SDS-PAGE has been used to perform the separation step. Further, the gel has been processed in the off-line molecular scanner mode by excising the sequence of partially overlapping spots. The MS-scanned profile of the gel has been reconstituted using the proprietary package 1D-ZOOMER. Each spot is identified either by MALDI-TOF fingerprinting or LC-MS/MS sequencing (or both). The collected data is assembled to produce the 1D proteomic map for the gel-separated proteins.

Profiles of individual proteins are extracted from the map and used to compute the cumulative protein abundance index, which is proportional to the number of identified peptides and to their reproducibility along the gel. As a result of procedure each sample is profiled with a list of identified proteins, where each protein is associated with an abundance index. Samples’ profiles were depicted on the plane using the Principal component analysis (PCA) statistical method. PCA arranges samples into the clusters according to the hidden resemblances of associated protein profiles. Preliminary results show, that there is a clear distinction between the control and treated samples, whereas PB and 3-MC cases differ only in strength of manifestation of these differences. Statistically this conclusion is derived from the 89% coverage of total variance by the first principal component. Contribution of different proteins into the recognizable patterns of PB and MC-induced toxicity in currently evaluated.

Optico-nanosensoric Protein Chips for the Registration of Protein Complexes

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Optico-nanosensoric protein chips are sensitive nanostructures with probe proteins immobilized onto them. Formation of specific protein complexes on the biochip’s nanostructure surface leads to an increase of the refraction index as is registered with the aid optical receivers.

In this study, the optical biochips to the two biosensor types have been fabricated: to CD-ROM biosensor and to “resonant mirror” optical biosensor. To fabricate CD-based biosensor, the protein adrenodoxin was immobilized onto the compact disk surface. The incubation of CD in solution containing the partner protein adrenodoxin-reductase, caused the formation of complexes on the CD surface which in turn led to the increase in the level of errors arising upon reading the information in the standard CD-ROM system. Application of biochips to CD-ROM makes these biochips attractive for use in proteomic studies. Another type of optical nanobiochips, fabricated on the basis of a “resonance mirror” optical biosensors, made possible the detection of disease markers in multicomponent mixtures. Onto the waveguide surface of this biosensor the antibodies to HBsAg and HCVcore Ag were immobilized. Such biochips were able to specifically capture the markers of infectious diseases—such as hepatitis B and—in the serum. The biochip with immobilized antibodies to serum amyloid A—the possible marker of oncodeseases—has been fabricated.

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High-performance Proteomics; Proteome Analysis of 1000 Microdissected Cells from Pancreatic Carcinoma Precursor Lesions


The decipherment of a proteome is straightforward approach to elucidate cell’s biology. But the identification of proteome changes underlying clinical pathogenic processes is often hampered by significant cellular diversity of the tissue. Pathogenic aberrant cells are surrounded by cells originating e.g. from stroma, the vascular system or other neighbouring cell types, which lead to under-representation of interesting cells when analysing whole tissue specimen. Therefore, selection of relevant cell types for detailed analysis is an absolute prerequisite for in depth elucidation of underlying biological processes. Microdissection offers the advantage to select for a biologically relevant cell type which is often in low abundance.

For the identification of molecular markers of the pancreatic tumour progression we established a proteomics approach allowing us to analyse microdissected cells stemming from pancreatic carcinoma precursor lesions. Due to the limited amount of proteins available from microdissection we developed a procedure including fluorescence dye saturation labelling in combination with high resolution two-dimensional gel electrophoresis. With this procedure we are able to analyse proteins extracted from 1000 microdissected cells with a high resolution of up to 2 500 protein spots. Using tissue arrays with tumour specimen from 130 patients we were able to successfully verify the identified candidate molecular markers by immunohistochemistry.

Proteomic Analysis of Nasal Aspirates Derived from Children Suffering Influenza Virus Infection


Respiratory infections caused by influenza are a major health problem worldwide. To date however, there are no studies showing the protein expression changes associated with influenza infections. Therefore, the aim of this study was to screen nasal protein expression for influenza virus respiratory infection using proteomics techniques. Nasal aspirates were obtained from children (n = 7) suffering cold infections and processed for virus detection and proteomic analysis. Control nasal samples were taken from the same group of children 8–10 weeks later when they were symptom free. Protein expression was analyzed using two-dimensional electrophoresis (2-DE) technique. Differentially expressed proteins in patients were identified using ESI-MS/MS (Q-ToF2). Thirty proteins were found to be differentially up-regulated in nasal aspirate samples from infected children compared with control samples. On the other hand, one protein was down-regulated in infected sample which was identified to correspond to lipocalin A. Our findings reveal that the composition of nasal secretions in influenza virus respiratory infections is different from that when children are healthy and may provide further insights into the pathogenesis of respiratory infections caused by influenza.

Sample Preparation for Plasma and Serum Profiling using Magnetic Bead Technology

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Serum and plasma peptide profiling using MALDI-TOF mass spectrometry (MS) is an increasingly popular approach used for the discovery of biomarkers and as a means to detect and diagnose disease and allow the assessment of disease severity, progression, and the effectiveness of treatments.

Before mass spectrometry can be used to generate serum/plasma peptide profiles reproducible, preferably automated, sample preparation procedures need to be used in which peptides are enriched and substances which interfere with MS analysis removed. Dynabeads® are uniform superparamagnetic monodisperse beads with a specific and defined surface for the adsorption/desorption and coupling of bioreactive molecules. We have developped ion exchange and reversed phase magnetic beads for serum/plasma peptide isolation. These beads enable large numbers of serum/plasma peptides to be processed and analysed simultaneously. The use of multiple bead surfaces for the capture of peptides from the same sample increases the number of peptides detected in a given sample and may increase the likelihood of discovering novel biomarkers. Sample preparation procedures have been successfully automated on robotic platforms.
Osmotic Stress Resistance in TALH-Cells

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Epithelial cells of the thick ascending limb of Henle’s loop (TALH) are normally exposed to variable and often very high osmotic stress as part of the urinary concentrating mechanism. The induction of ER stress proteins is a part of the TALH-cells reaction to NaCl osmotic stress. In order to map the expression changes of ER stress proteins under different stress factors and to understand the role of this protein in osmotic stress resistance, we performed differential proteomic analyses with established TALH model cell lines for three different stress factors.

The exposure of TALH-cells to hyperosmotic stress led to a complex reaction which was reflected in alteration in the expression of a large number of proteins belonging to different functional groups. The ER stress proteins especially GRP78, GRP94, calreticulin, and Erp72, represent one of the protein groups that were altered with osmotic stress. Exposing TALH-cells to higher NaCl concentrations resulted in a downregulation of GRP78, GRP94, calreticulin, and Erp72. Similar effect was also observed for the calcium binding protein calreticulin when the cells were exposed to high concentration of urea and glucose. The glucose regulated proteins GRP78 and GRP94 were only downregulated in case of NaCl, but not in case of urea or glucose stress. 2D-GE and Western blot analysis show the two forms (oxidized and reduced) of the protein disulfide isomerase Erp72 by TALH-cells. NaCl and glucose caused stress resulted in the downregulation of one of the forms, whereas under urea stress both forms could be detected. Immunofluorescence staining shows that in case of NaCl stress the downregulation of Erp72 is accompanied by a translocation of the protein to the nucleus. With our investigation we shed light on the multifaceted role of the ER stress protein in the reaction of the TALH-cells to the hyperosmotic stress.
Development of Ultra High Performance Multi-dimensional Protein Identification Technique and Its Comparative Proteomics Application in Alzheimer Model Mouse Brain Tissue

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Shotgun proteomics approach became a method of choice for protein mixture analysis due to its simplicity and rapid analysis speed. Shotgun proteomics approach has benefited greatly from recent advances in capillary scale multidimensional peptide LC separation techniques. Yates and his coworkers at Scripps Research Institute developed a well-known proteome analysis technique, multi-dimensional protein identification technique. It uses single capillary column constructed with a serial packing of strong cation exchange and C18 reverse phase column material to fractionate and separate peptides sequentially. Protein profiling analysis of many biologically important samples are routinely carried out with MudPIT technique. One limitation of this technique is that separation capability of conventional capillary RPLC is not sufficient enough for the direct analysis of whole tissue protein extract samples.

In this study, we introduce a newly developed ultrahigh performance multi-dimensional protein identification technique which combined a ultra high pressure RPLC system (operating pressure of 10,000 psi) with a MudPIT system. Significant improvement in terms of peptide detection sensitivity and peak capacity of peptide LC separation was made over conventional MudPIT system. A very complicated proteome sample, yeast whole cell lysates was investigated to compare the performance of UHP-MudPIT with other multidimensional peptide separation techniques. As a comparative proteomics application, whole brain tissue proteome samples from normal and Alzheimer model mouse were analyzed by the newly developed UHP-MudPIT system. In this mouse brain proteome study, we were able to identify many new proteins, previously undetected by conventional MudPIT system, and to observe dynamic changes of protein abundances between two brain tissue samples. It appears that our newly developed UHP-MudPIT system has enough potential to become a target protein screening system or a disease-specific biomarker discovery platform by achieving precise determination of in-depth protein profiles and quantitative analysis of different proteome samples under various patho-/biological conditions.

Improvement of Urine Protein Expression Profiling with SELDI (Surface Enhanced Laser Desorption Ionization): Comparing Different Sample Preparation Techniques

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Aims: Disease diagnosis and monitoring in urology is still based on invasive and tissue based approaches. However, urine represents an easily accessible body fluid that can be used for highly sensitive and highly specific proteomic analysis. We compared various protocols for SELDI-MS to improve the protein expression profile according to peak number, peak intensity and peak FWHM.

Methods: Midstream urine from healthy persons was investigated by SELDI-MS using cation (CM10) and anion (Q10) exchange chips. Different ways of sample preparation (centrifugation, dilution, and precipitation) as well as washing/binding buffers, and various matrices (sinapic acid (Fluka), sinapinic acid (Sigma and Ciphergen), cinnamic acid, (Sigma and Ciphergen), and combinations thereof were tested. Samples were analyzed on a PBS II c ProteinChip Reader (Ciphergen) using laser settings of 125, 150 and 175 units.

Results: The use of CM10 chips leads to higher peak numbers and higher peak intensities, compared to the corresponding anion exchange chips Q10. At lower laser intensities, cinnamic acid matrix from Ciphergen on CM10 chips results in highest peak numbers, highest peak intensities and smallest peak FWHM. Cinnamic acid matrix from Sigma comes second in this comparison. On Q10 chips a binary mixture of sinapinic acid and cinnamic acid (both Sigma) gives better results than the pure matrices. Using higher laser intensities may lead to higher total peak intensities but leads to significantly higher FWHM values, i.e. lower resolution.

Conclusions: The impact of different matrices on the quality of SELDI-MS spectra (peak number, peak intensity, resolution) is found to be large. In order to generate meaningful, clinically relevant information, establishing a protocol that results in reproducible, stable, and high quality urine SELDI mass spectra is a must.
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SELDI-TOF MS for Rapid Diagnosis of Ascites in Patients with and without Peritoneal Carcinosis
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Background: Ascites is one important body fluid used for tumor diagnosis (peritoneal carcinosis). The routine method to discover malignant peritoneal processes is a routine cytology assessment of ascites with the microscope. This method is time consuming and cells detected therein are changed morphologically. Especially in ovarian and pancreatic cancer a lot of effort is put into early diagnosis and valid screening methodologies, but yet there is no published data on protein screening in ascites.

Methods: Ascites samples were collected, categorized by patient history and cytological screening. We applied samples of patients with and without the presence of cancer to SELDI (surface enhanced laser desorption/ionisation) mass spectrometry (Ciphergen, CA, USA) using optimized protocols for Q10 surfaces. For a preliminary test we analyzed eight samples of each group. Following 5 min pre-incubation with binding buffer (10mM ammonium acetate, 0.1% Triton X100) the diluted samples (5 μl sample + 45 μl binding buffer) were incubated onto the chip. After air drying, matrix (CHCA saturated in 50% ACN/50% water/0.5% TFA) was applied (2x 0.8 μl). Data were analyzed using the ProteinChip® (Ciphergen), XLminer (Biocontrol, Jena, Germany) and ClinProTools 2.0 (Bruker, Bremen, Germany) software, respectively.

Results: Data suggest for the Q10 chip a very distinct pattern for the malignant (positive) ascites vs. benign (negative). This is true for all software approaches used, thus emphasizing the impact of our findings. With the software used, we found a specific peak around 14 kDa (Ciphergen), a good prediction according to Clustering (Biocontrol) and a classifying pattern of five peaks (4476–16673 Da) (Bruker).

Conclusion: With our preliminary results the two patient groups could be distinguished, clearly. The implementation of SELDI in protein profiling for cancer diagnosis in ascites is of potential clinical value.

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Optimization of Reverse Phase Protein Microarrays for Blood Biomarker Validation
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Reverse phase protein microarrays (RPPMs) are a promising tool for biomarker validation. With this protein microarray format, only minute amounts of clinical material are printed per spot and a large number of clinical samples can be screened simultaneously for a marker of interest using validated antibodies. The use of serum and plasma in this array format has great clinical potential considering the large number of putative biomarkers present in blood. Few studies have reported the use of biological fluids such as plasma or serum to print RPPMs, and all have used different protocols to manufacture these arrays. The standardization of protocols for RPPM construction will help in the comparison of results between different laboratories using this type of sample and technology. In the present study we printed serum and plasma RPPMs with different protocols (substrates and buffers) previously reported to be used for blood samples. To compare the different results and identify the protocol best suited for printing biological fluids, arrays were probed with an antibody against clusterin, a putative cancer biomarker whose detection was already validated in plasma and serum using RPPMs. We performed a series of experiments using recombinant clusterin spiked in plasma in order to compare RPPMs with an ELISA kit. Our analysis showed excellent correlation ($r^2 = 0.96$) between the spiked clusterin levels estimated with the ELISA and the RPPM. In order to test the robustness of our platform we spotted 200 clinical samples (serum and plasma) on RPPMs. We report the results from analysis of replicates, dye swaps, and the effects of sample freeze and thaw cycles on clusterin levels. Finally, we compared data normalization with a control antibody and an antibody against human transferrin. RPPMs appear to be a reliable and reproducible technology for the high-throughput validation of biomarkers present in human blood samples.
MALDI-TOFMS High-density Polymer Micro Array for Direct Protein Analysis from Complex Biological Samples

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Recently, nonmetallic disposable MALDI sample platforms have been introduced as the cost-effective alternative for conventional metallic target plates. In the following sections, we suggest a new approach to prepare plastic MALDI chips and reporting the first performance test on diverse sample set using a vast polarity/hydrophobicity range accessible by our new fabrication protocol.1

An atmospheric molding protocol has been used to prepare hydrophobic and ionic methacrylate-based copolymer pMALDI-MS chips by targeting selected groups of various monomers copolymerized during molding, namely, alkyl (C1-C18), carboxy, sulfo, dimethylalkylamino, and trimethylalkylammonium. The new disposable array chips provide analyte-oriented enhancement of protein adsorption to the modified substrates without requiring complicated surface coating or derivatization. Efficient desalting of a plant Δ-11-desaturase membrane protein fragment and of human plasma was performed on a poly(butylmethacrylate-co-methylmethacrylate) copolymer array by simple washing. The MALDI-MS performance of ionic copolymer chips was evaluated for lysozyme, β-lactoglobulin A, trypsinogen and carbonic anhydrase I using washing with solutions prepared in pH or ionic strength steps. On cationic chips the proteins are washed out at pHs lower than their pI values, and on anionic chips at pHs higher than their pI values. The ability of the microfabricated pMALDI chip set to selectively adsorb different proteins from real samples and to significantly increase their MS-signal was documented for the transmembrane Photosystem I protein complex from the green alga Chlamydomonas reinhardtii. Novel pMALDI targets show a favorable performance for analysis of proteins and peptides containing salts, detergents, and other contaminants. Latest development is focused on functional assay of unknown proteins followed by on-pMALDI-chip trypsin-mediated digestion and PMF characterization of the enzymatic active protein(s) in the sample. Reference


Proteomic Signatures Corresponding to the Histological Differentiation in Lung Cancer

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Histological differentiation in lung cancer is highly correlated with the malignant potential of tumor cells, the response to the chemotherapy, and the prognosis of the patients. However, the molecular background of lung cancer differentiation is largely unknown and few numbers of proteins was reported as potential tumor marker for differential diagnosis. The understanding of the proteomic background of lung cancer histology, and the identification of individual proteins, the expression which is highly specific to the certain histological type, will facilitate the establishment of novel therapeutic strategy for lung cancer.

We conducted proteomic studies on lung cancer. We used surgical specimens from 85 cases of lung cancer with different histological subtypes including adenocarcinoma (13 cases), squamous cell carcinoma (19 cases), small cell carcinoma (10 cases), large cell neuroendocrine carcinoma (LCNEC)(32 cases), and typical or atypical carcinoid (11 cases). We also used 34 lung cancer cell lines with different histological origin. Protein expression profiles were produced by two-dimensional difference gel electrophoresis (2D-DIGE) with a large format gel, and the intensity of approximately 3,000 spots was converted to the numerical data for the expression study.

Unsupervised classification separated the samples into two groups: in vitro cell lines and clinical specimens. Data-mining approach identified the specific clinico-pathological parameters that were highly associated with the overall feature of proteome as well as a small number of the key protein spots relevant to the histology. These proteins will be good candidates for tumor markers and therapeutic targets.
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Intensive Proteomic Approach to Identify Secreted Peptide/Protein from 3T3-L1 Adipocyte and Primary Adipocyte and the Influence of Thiazolidinediones on Secretome


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Like an endocrine organ, adipocytes have been shown to secrete a number of important secretory molecules as a adipokine with a role in energy metabolism, reproduction, cardiovascular function and immunity. Many adipokines such as leptin, TNFα, adiponectin and resistin have been identified from adipose tissue related in obesity/type 2 diabetes, and the secretion of these hormones is tightly regulated by other cytokines and nutritional status. Furthermore, thiazolidinediones(TZD) exert their antidiabetic effects through a mechanism that involves activation of PPARγ altering the transcription of several genes involved in glucose metabolism and energy balance. In this study we have approached to identify new secretory peptide/protein from adipocytes using primary adipocyte and differentiated 3T3-L1 adipocytes and examine the influence of TZD on the regulation of peptide/proteins secretion from adipocyte. One culture plate was left in serum-free medium and the other was serum-free medium with rosiglitazone, a TZD, for 16h. The supernatant was harvested and lyophilized. In the case of primary adipocytes, the cell epididymal fat of 6 weeks old male rat was incubated in rosiglitazone treated and non-treated KRH buffer for 6h. After desalting and purifying using C-18 cartridge, all secretome samples were fractionated by RP-FPLC column. Separated fractions were trypsin-digested and analyzed by nano-LC MS/MS. From differentiated 3T3-L1 adipocyte secretome we identified 279 of proteins. 75 of those data.

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An Interaction Map of Chinese Liver 26s Proteasome Subunits

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The 26s proteasome is a multi-subunit protein complex in eukaryotic cells. It is involved in an important protein degradation pathway related to the regulation of cell cycle, cell apoptosis, antigen presentation, gene transcription and so on. The 26s proteasome complex is composed of two subcomplexes, the 20s catalytic particle and the 19s regulatory particle. The 20s particle has a cylinder-shaped structure containing four stacked heptameric rings that form the proteolytic chamber. The 20s cylinder is capped on both sides by a 19s particle to form the 26s proteasome. The 19s particle is composed of at least 17 proteins that form two subcomplexes; the “base” complex is composed of six AAA family of ATPases and three other proteins, and the “lid” complex is composed of eight non-ATPase proteins. It is generally believed that the 19s particle plays two major roles in facilitating protein degradation by the 20s particle: the first is to recognize and position ubiquitinated protein substrates, and the second is to unfold the protein substrates so that they can be inserted to the proteolytic chamber of the 20s catalytic particle.

Human liver is one of most important organs. Protein degradation plays a very important role to maintain normal physiological functions in liver. To understand proteasome and its related protein degradation pathway we cloned ORFs of 34 proteasome subunits from human liver and detected their interactions between these subunits by approaches such as yeast two-hybrid system, GST pull-down and subcellular localization. In the present observation, there are 112 interacting pairs found in proteasome complex. 34% of our observed 26s proteasome interactions have been reported previously in various systems. Those novel interactions include interactions between 7 subunits and 19s RP subunits. It is with interest to characterize those interactions and predict the 26s complex structure with those data.
A Strategy for the Plasma Proteome Research Based on Stable Isotope Labeling and High Accuracy FTICR MS

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A new strategy for the establishment of plasma proteomics core data combining immunoaffinity separation, acetic anhydride stable isotope labeling and high accuracy FTICR mass spectrometry with in-house data analysis software ASMQ was developed.

Firstly, the top-12 high abundant proteins in human plasma were depleted with the immunoaffinity fractionation column and the low abundant proteins were enriched in the flow-through fraction (FF). Secondly, the proteins in FF fraction were digested by trypsin and then split equally and labeled by stable isotope reagent—d0/d6 acetic anhydride (1:1) respectively. As a result, the labeled peptides derived from plasma proteins will display a pair of peaks with 3.0 Da mass shifts (single charged) in the mass spectrum, which will result in an enhancement of the positive recognition capability. Thirdly, after multi-dimensional chromatography separation, the eluted peptides were identified with a high accuracy (<5 ppm), high dynamic range (single spectrum >5000) and high sensitivity (sub-fmol) LTQ-FT mass spectrometer. Lastly, the MS data was searched against human IPI database with local Mascot software. Then the search results (p < 0.05) were associated with MS original raw file using in-house software ASMQ. All the observed peak ratio of the labeled peptides could be calculated and compared with the theory value equal to 1.0. Through the statistical analysis of normal distribution fit, the 99% confidence interval was defined. Analysis on the sample with the immunodepleted only but no further prefractionation generated 959 quantified proteins and 1624 quantified peptides in a single LC-MS/MS run. The average calculated ratio was 1.04, and the 99% confidence interval was from 0.67 to 1.33. The experimental result showed that the strategy developed by the research has the tremendous potential to provide a deeper coverage of human plasma proteome.

One-step On-plate Desalting Protocol for MALDI-MS; Using Hydrophobic Polymer Micro-contact Printing Technique

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Matrix-assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS) has become one of the most important technologies in proteomics. However, in the presence of detergent or salt, the sensitivity and reproducibility of MS could be worse. The identification of trace peptides become extremely difficult resulting mainly from various problems, such as the uncontrollability of the spot size, the deficient homogeneity of matrix crystallization, and the limited ability to resist contamination.

To resolve better the mentioned existing problems, we explore a novel one-step on-plate desalting (OPD) protocol for MALDI-TOF-MS by using hydrophobic polymer micro-contact-printing technique. Small hydrophobic spots of linear polymers were patterned in the centers of every MALDI metal plate wells firstly. Tryptic-peptide solution with no pre-desalting was dropped onto the central hydrophobic spots, resulting in a concentration of peptides on the hydrophobic polymer surface with a reduced spot size. The dried peptide layer was then covered subsequently with over-volume matrix solution, causing of an automatic removing of redissolved salts from the spot center to the spot edge by means of a natural “outward flow.” The desalting and peptide enrichment can be achieved on prestructured MALDI target with no additional water rinsing step. Four sorts of linear polymer—polymethyl methacrylate (PMMA), PMMA derivatized with Fullerene (C60) (PMMA-C60), polystyrene (PSt) and PSt derivatized with C60 (PSt-C60)—were selected as the layer material coated on MALDI target. The OPD protocol exhibited a dramatic enhancement in signal-to-noise ratio up to 850 for 14 fmol BSA digests in the coexistence of 100 mM salts, compared with barely detectable peaks in traditional way. Finally the novel OPD protocol was applied to identify proteins of human liver tissue. The real sample analysis has showed that the success rate of identification was increased by 2-fold for low abundance proteins than that by a conventional ZipPlate desalting strategy.
High Confidence and High Accuracy Data Analysis Based on FTICR Mass Spectrometer in Large Scale Human Liver Proteomics

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A hybrid mass spectrometer LTQ-FT, combining high scan rate of tandem mass spectrometry of the linear ion trap with the high resolution /mass accuracy of the FTICR, was used in human liver proteomics research. Proteins extracted from normal human liver were digested, separated by offline SCX and online RP, and analyzed by LTQ-FT.

To observe and evaluate the performance of the instrument, three MS scan modes were performed: (1) LTQ with normal scan mode; (2) FT scan mode without SIM scan; (3) FT scan mode with SIM scan. Two algorithms, SEQUEST and Mascot for database searching were also utilized and compared in the experiment.

Distribution of Xcorr and \( \delta \text{Cn} \) value under three MS scan methods was compared, and the impact of different mass accuracy setting on database searching was investigated. The result indicated that high mass accuracy MS data enhanced the confidence of identified peptides by increasing the \( \delta \text{Cn} \) value and decreasing the Xcorr value. Comparing the throughput of peptides and proteins identified among three MS scan methods, we found that FT scan with SIM scan identified the least peptides and proteins, whereas the LTQ with normal scan and FT scan without SIM made similar throughput on peptides and proteins identified. To evaluate the database searching result by Mascot with high accuracy data, the target-decoy database searching strategy was used and the peptides with mowse score of more than 30 were selected to guarantee the same false positive level with SEQUEST. The result showed that Mascot and SEQUEST had similar yield for high accuracy MS raw data.

Based on the research above, a high confidence and high accuracy dataset of human liver proteome was developed with 4935 unique proteins identified with the false positive rate less than 1% in peptide level and 1.5% in protein level.

Proteomic Analysis of Human BALF; Comparison of Bronchial Asthma with Normal Control


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Bronchoalveolar lavage fluid (BALF) is presently the most common way of sampling the components of the epithelial lining fluid and the most faithful reflect of the protein composition of the pulmonary airways. Soluble proteins in BALF might originate from a broad range of sources, such as diffusion from serum across the air-blood barrier and the production by the different cell types. In this study, we had compared the BALF protein profiles of bronchial asthma patient \((n = 8)\) and healthy normal subjects \((n = 8)\). The airway protein pattern changes in bronchial asthma BALF were analyzed by 2-DE.

Twenty proteins were found to be differentially expressed in BALF between asthma patients and normal healthy control. These proteins, which were identified by matrix assisted laser desorption ionization time of flight (MALDI-TOF) MS, including Vitamin D binding protein, Hemopexin, and Haptoglobin \( \beta \), 10 proteins were up-regulated whereas 10 proteins including \( \alpha 1 \) antitrypsin, apolipoprotein AI and aldose 1 epimerase were down-regulated. Among them, Vitamin D binding proteins levels in BALF were validated by using ELISA. The concentrations of vitamin D binding protein were higher in asthma patients \((n = 52)\) than in normal control \((n = 14)\).

Identification of proteins in the BALF and their expression changes at different stages of asthma could provide further insights into the complex molecular mechanisms involved in this disease. A large portion of these proteins and their expression changes were identified for the first time from BALF, thus providing new insights for finding novel pathological mediators and biomarkers of asthma. These results suggest the involvement of the vitamin D binding protein in the pathogenesis of bronchial asthma.

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Toward Comprehensive Expression Proteomics in Cancer Research

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To develop the better therapeutic strategy for cancer patients, we are conducting proteomic study. Our research goal is 1) to gain a comprehensive understanding of the proteomic background which governs the cancer biology, 2) to identify the proteins which essentially play a key role in cancer phenotypes including histology, metastatic potential, and response to the existing therapy, and 3) using the identified proteins, to develop the practical tumor markers for the personalized medicine. Presently, we are investigating four types of malignant tumors: liver cancer, lung cancer, esophageal cancer, and soft-tissue sarcoma.

In our study, 2D-DIGE with a large format 2D system generates the protein expression profiles using the surgical specimens and plasma samples. For accurate proteomic study, specific population of the cells is recovered by laser microdissection. To reduce the complexity and dynamic range of plasma proteins, the plasma proteins are separated by multi-dimensional liquid chromatography prior to 2D-DIGE. Multiplex detection system with the internal control in 2D-DIGE allows the quantitative proteomic study with a less labor intensive way. The proteome data is converted to the numerical ones, and the data-mining approach captures the proteomic signature corresponding to the important clinico-pathological parameters. Mass spectrometry identifies the proteins corresponding to the protein spots. The proteins involved in the signature will be candidates for tumor markers and therapeutic targets. The monoclonal antibody is developed against the identified proteins for further functional study and development of clinical application. The RNA expression level is also monitored by GeneChip using the identical frozen tissues.

We identified the proteins associated with 1) response to the tyrosine kinase inhibitor in lung adenocarcinoma, 2) prognosis of the patients with soft-tissue sarcoma, 3) lymph node metastasis in esophageal cancer, 4) early recurrence in liver cancer, and 5) histological differentiation of lung cancer. Their clinical applications are under investigation.

Protein Expression Differences between Der p1 Positive and Negative Asthma Patients Plasma

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Der p1, a cysteine proteinase derived from the house dust mite (HDM) Dermatophagoides pteronyssinus, is a major component of the allergic immune response in HDM atopic asthma patients. However the role that house dust mites play in the primary causation of asthma is controversial. To better understand AIA, we adopted a proteomic approach. Twelve six patients with bronchial asthma were recruited for this study. Each patient underwent pulmonary function testing before and after DP challenge. The patients with more than a 15% fall in FEV1 following DP challenge was defined as positive group. The plasma of 26 patients with negative and positive response to DP was obtained before and 4h after DP challenge. The discrete proteins of these two groups were displayed by 2-dimensional electrophoresis (DE). The 2DE image disclose the difference between DP challenged negative (n = 6) and positive (n = 6) groups on spot intensity patterns.

At baseline, six proteins differed in the quantity between the two groups. The relative intensities of Fibrinogen fragments were significantly lower in DP positive group than negative group. In contrast, the intensities of complement C3 and C3d were higher in DP positive group than negative group. After antigen challenge, five proteins including transthyretin were differently expressed between the positive and negative groups. The change of intensity before and after DP antigen challenge was calculated as the intensity of spot after over before DP challenge. The changes of relative intensities of complement C3 decreased while, the ratio of complement C3d, C4a and Apo E3, transthyretin and PRO2675 significantly increased in the DP positive group when compared to negative group. In conclusion, DP antigen induced asthmatic shock may be related to alterations in the levels of complement, as well as those of lipoprotein and other proteins.
Defining the Human Plasma Proteome with PeptideAtlas
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Human plasma is thought to contain many different proteins, perhaps nearly all human proteins on account of low-level tissue leakage. The protein content of human plasma is thus considered important for medical diagnosis and has the potential to provide a complete snapshot of the health of an individual. Tandem mass spectrometry is a powerful method for developing an understanding of the content of human plasma, but a single MS/MS study generally only observes a small fraction of the plasma proteome. Here we present the Human Plasma PeptideAtlas, a compendium of all peptides observed from over 40 different experiments analyzing human serum and plasma samples contributed from many different labs.

All 12 million tandem mass spectra from nearly 40000 mass spectrometry runs were searched using SEQUEST, and then each putative identification was assigned a probability of being the correct identification using the PeptideProphet software. The results of this automated searching and validation with an error model were loaded into the SBEAMS–Proteomics database. All peptides with a PeptideProphet probability \( P > 0.90 \) were combined in the database to form a master list of observed peptides across all these experiments. This list of peptides was then mapped to the Ensembl human proteome and genome, and the results are loaded into the PeptideAtlas database.

We identify over 18000 distinct peptide sequences more than once, mapping to over 2500 proteins (after reduction for multiple mappings), corresponding to about 15% of known genes.

The results of this process are publicly available at http://www.peptideatlas.org in the form of downloadable peptide lists as well as a database interface that can be used to browse the atlas at both the protein and peptide level. This atlas is a valuable resource for planning targeted proteomics experiments and as an aid in analyzing new plasma samples.

Automatic Off-line Enrichment of Phosphopeptides Using Titanium Dioxide
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Mass spectrometry-based elucidation of many cellular processes such as signal transduction requires the qualitative and quantitative identification of phosphorylation events. Several approaches are used to enrich phosphorylated peptides based on ion-exchange, ion-chelating or chemical reactivity of the phosphate group as well as phosphospecific antibodies. These approaches often require chemical modification of tryptic peptides or have limited selectivity. Recently, titanium dioxide has been introduced as a powerful alternative for the enrichment of phosphopeptides based on the selective coordination of the phosphate group to titanium.

In quantitative side-by-side comparisons using stable isotope labeling of peptides (iTRAQ) we show that titanium dioxide affords about 60% higher recovery for singly phosphorylated peptides and 5–10 fold higher recovery for doubly phosphorylated peptides compared to IMAC. In addition, titanium dioxide is less sensitive to salt present in a sample than IMAC. As a result, fractions from strong cation exchange chromatography can be applied directly to the column without prior desalting.

In order to make this approach compatible with high throughput proteomic workflows, we have developed an automatic off-line system for routine separation and analysis of phosphorylated and non-phosphorylated peptides from complex protein mixtures in a 96 well format. This effectively adds an additional peptide separation dimension that can be easily implemented in conventional GeLC-MS and MudPit workflows.
Analysis of cGMP/cAMP Signaling Pathways Using Chemical Proteomics

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To tackle dynamic range problems in the analysis of complex proteomes, sub-proteomic approaches have been developed. Immobilized small molecules, for example, can be used to specifically enrich for proteins that show affinity for the molecule. Our interest focuses on protein-cyclic nucleotide (cyclic AMP (cAMP) and cyclic GMP (cGMP)) interactions, as these secondary messenger molecules play essential roles in numerous signalling pathways. Protein kinase A, the primary target of cAMP, is compartmentalized in cells and tissue through interaction with so-called A-kinase anchoring proteins (AKAPs). This makes the cell- and tissue-specific identification of AKAPs of great interest.

cAMP and cGMP were immobilized onto agarose beads. Optimization of affinity pull-down procedures in mammalian HEK293 cells revealed that a large number of proteins were pulled down using the cyclic nucleotide beads. Protein identification using a LTQ FT-ICR-MS/MS showed that many were indeed genuine cyclic nucleotide-binding proteins. In addition however, several of the pulled-down proteins were general nucleotide or DNA/RNA-binding proteins. Consequently, a sequential elution protocol was developed in which solutions of ADP, GDP, cGMP and/or cAMP were used to selectively elute proteins binding to these nucleotides. The fraction remaining on the beads showed a specific enrichment, not only for cAMP/cGMP binding proteins, but also for their interactions partners, like AKAPs.

Subsequently, the protocol was used to investigate the “cGMP/cAMP interactome” in rat heart tissue. This not only showed enrichment of the kinases PKA and PKG and several phosphodiesterases, but also of 8 known and 2 novel AKAPs. Amongst the latter was the sphingosine kinase type1-interacting protein (SKIP), recently proposed to be a potential AKAP. Our approach combined with extensive bioinformatics approaches reveals that SKIP is indeed a genuine AKAP, highly abundant in heart tissue.

We are currently exploiting this protocol to study cGMP/cAMP signalling pathways in undifferentiated and differentiated embryonic stem cells.

Preparation and Characterization of Monoclonal Antibodies against High Abundant Proteins in Human Parotid Saliva

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Oral fluid is a perfect medium to be explored for monitoring health status, disease onset and progression, and treatment outcome through noninvasive means. Thus, human saliva has great diagnostic potential not only for oral, but also for systemic diseases. The purpose of the present studies was to prepare and characterize monoclonal antibodies (mAbs) against high abundant proteins in human parotid saliva for the depletion of these high abundant proteins in the future proteomic studies of saliva. Proteins in human parotid saliva were concentrated by using ultrafiltration and analyzed by SDS-PAGE. The proteins strap between 50–65kDa was cut, grinded and used to immunize BALB/c mice. The mAbs against high abundant proteins in human parotid saliva were prepared through hybridoma technology and characterized by ELISA and Western blot. Ten hybridomata cell lines secreting mAbs against high abundant proteins in human parotid saliva were established, and Western blot assay showed that these antibodies were specific for the high abundant proteins in human parotid saliva. Importantly, mAbs against salivary amylase, the most abundant protein in human parotid saliva, were characterized by ELISA. We conclude that mAbs against human high abundant proteins in parotid saliva were successfully prepared and characterized. The present studies may provide a premise in the application of these mAbs, which was to remove high abundant proteins in human parotid saliva, and better enrich and visualize low abundant proteins for the studies of disease-related biomarkers in human saliva proteome.
Differentially Expressed Proteins Induced by IL-3 in B-Lymphocytes


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A glycoprotein hormone interleukin-3 (IL-3) has an important role for growth of hematopoietic progenitor cells. IL-3 exerts its biological functions through a specific receptor (IL-3R) that is expressed on its target cells. The IL-3R activates multiple signal transduction pathways resulting in the induction of immediate early genes. To gain an insight into molecular mechanisms underlying the control of morphogenetic signals by cytokine induction, we used two-dimensional polyacrylamide gel electrophoresis (2DE-PAGE) and other proteomic approaches to identify proteins expressed in a murine pro-B cell line (Ba/F3) in response to the cytokine. Proteins were extracted from Ba/F3 cells after stimulation with IL-3. The proteins were subjected for separation by 2-DE. Out of induced proteins by IL-3 treatment, eleven proteins were analyzed by matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) and LC-MS/MS. Out of eleven proteins induced, COP9 signalosome subunit 4, Arhgdib and Ywhag proteins were highly expressed by the IL-3 induction: these have not been reported previously in Ba/F3 cells. The expression levels of these proteins were examined by Western blotting with the respective antibody to assess the protein values and by comparing mRNA expression levels in the same samples. It clearly suggests that the comprehensive approach to reveal the IL-3 stimulated protein profile and these results altogether provide the insight into elucidating role of IL-3 on the growth and differentiation of hematopoietic progenitor cells.

ECL Plex Multiplex Detection of Low Abundant Proteins in Cell and Tissue Samples

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The ECL Plex® Western blotting system comprises CyDye conjugated secondary antibodies, low fluorescent membranes and fluorescent molecular weight markers. Together with an optimal blocking agent, high performing imagers and analysis software, this system enables reliable multiplex detection of low abundant proteins with high sensitivity, linearity and dynamic range of nearly four orders of magnitude. Enhanced chemiluminescence western blotting combined with detection on X-ray film or CCD camera is sensitive, but offers a limited dynamic range and quantitative ability, due to protein loss caused by stripping and re-probing. The ECL Plex system is the first choice of method for investigating activation status of low abundant endogenous proteins in different cell signaling systems with accurate relative quantifications.

Using the ECL Plex fluorescent based western blotting system we have been able to study proteins involved in different signalling pathways that regulate cell growth, survival, actin reorganisation, migration, differentiation and apoptosis, in response to fibroblast growth factor-2 (FGF-2) and transforming growth factor β (TGF-β). We could detect different levels of ApoE together with the house-keeping protein β-Tubulin in different areas of control and transgenic mouse brain (cortex, hippocampus, and thalamus). Expression of total ERK1/2 (phosphorylated and non-phosphorylated) in response to FGF-2 stimulation of wild type (control) and C5-epimerase (enzyme involved in biosynthesis of heparan sulphate) knockout mouse embryonic fibroblasts. Embryonic mouse fibroblasts lacking the C5-epimerase showed increased levels of total ERK 1/2 relative to the house keeping protein GAPDH. Human prostate cancer cells (PC-3U) showed a biphasic activation of phospho-Akt relative to total Akt in response to TGF-β stimulation. The ability to quantify the levels of low abundant protein of interest in all these different cell systems relative to a house-keeping protein made it possible to see changes in protein levels, not seen previously.

*Disclaimer: ECL Plex and CyDye are trademarks of GE Healthcare.
AFM Fishing Is the Way to Reverse Avogadro Number in Proteomics

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Proteomics enables to identify and analyze proteins with a high throughput. Methodically, proteomics is based on combination of knowledge about genome with latest achievements in mass-spectrometric technology; such combination allows rapid proteins identification in a biological sample. But proteomics lacks a method for multiplication of individual molecules similar to the PCR-based one. So, proteomics is limited by concentration sensitivity level (CSL) of existing technologies which are unable, despite the successful development of mass spectrometry, 2D-electrophoresis, multidimensional chromatography and a variety of other methods, to overcome the CSL of 10–12 M. Why CSL is very important in electrophoresis, multidimensional chromatography and a variety of other concentration-sensitivity-level techniques which are limited due to the reversibility of binding. In case fishing becomes irreversible its combination with AFM detector enables to register single protein molecules and that opens up a way to lower concentration sensitivity limit down to the reverse Avogadro number usage of AFM. Possibilities of AFM biochips application for anti-HCV core revelation at the concentration about 10–17 M were demonstrated. AFM biochips for registration of hepatitis B and C markers from patient serum were created.

Proteome Analysis of Transcription Factor Complexes during the Mammalian Cell Cycle

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We performed an in-depth proteomic study to quantify dynamic relative protein expression patterns of TATA binding protein (TBP) containing complexes during the cell cycle. TBP plays an essential role in the regulation of transcription by all three nuclear RNA polymerases and is known to be present in different protein complexes.

Double Affinity Purification was used to purify complexes containing TBP from human HeLa cells. Flag-HA purification of tagged TBP from HeLa cells resulted in the successful identification of all known stable interactors from the TBP complexes TFIIA, B-TFIIA, SL-1, TFIIIB and TFIIID. Moreover, novel post-translational modifications, like phosphorylation, acetylation, and methylation of TBP and its interactors were identified as well as putative novel interactors of TBP at substoichiometric levels.

To analyze the dynamics of protein complexes during the cell cycle, we performed a quantitative proteome analysis using SILAC labeling in synchronized cell cultures. HeLa cells were synchronized at G2/M transition or at the G1/S transition using nocodazole or a double thymidine block, respectively. The cells were then released from the block and samples were taken at different time points after the release. TBP complexes were isolated from the samples and analyzed by mass spectrometry. Results of this will be discussed.

In summary, by integrating double affinity-purification, metabolic stable isotope labeling and mass spectrometry, we obtained insight into cell cycle dependent regulation of crucial transcription factor complexes.
Proteomic Analysis of Cancer of Buccal Mucosa; Tissue Protein Profile and Antibody Response to Tumor Antigens

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Oral cancer is the sixth common malignancy and a major cause of cancer morbidity and mortality worldwide and buccal mucosa is one of the most commonly affected sites. This is also the major cancer presenting in males in India. The five-year survival is low and about 50% return with a recurrence or nodal metastasis. The choice of therapy, detection of nodal invasion or prediction of recurrence is based on clinical and histopathological observations, and previous clinical experience. However, disease that is clinically similar can occur due to diverse combinations of molecular alterations, which also determines disease prognosis. We have therefore used proteomics to obtain molecular signatures for squamous cell carcinoma of the buccal mucosa.

In this study, proteins which differentiate between microdissected epithelium from tumor and adjoining non-transformed tissue, have been short-listed by PDQuest analysis of protein spots separated by 2D-gel electrophoresis and confirmed by statistical and cluster analysis. The expression of the proteins altered in cancer has been assessed by western blotting. In a complimentary approach, tumor antigens which elicit an antibody response in cancer of buccal mucosa, have been identified using immunoproteomics. Sera from patients and controls were screened against a protein profile of an oral epithelial cell line. Antigens detected by the patient’s sera were different among individuals with presence of any single antigen ranging from 10–80%. The identity of the proteins was obtained by mass spectrometry and they include members of metabolic pathways, cytoskeletal proteins and chaperones. One of the metabolic enzymes eliciting an antibody response is also increased in expression in cancer of buccal mucosa.

The two approaches have complimented to broaden the battery of protein markers that are available to form a molecular signature for squamous cell carcinoma of buccal mucosa.

GroES, a Novel Virulence Factor of Helicobacter pylori; Roles in Inflammation and Gastric Carcinogenesis

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Helicobacter pylori is a major risk factor of gastric cancer. Although many H. pylori virulence factors have been reported, the pathogenic mechanism by which H. pylori infection causes gastric cancer remains unclear. The aims of this study were to identify gastric cancer-related antigens from H. pylori and characterize their roles in the development of gastric cancer. Using proteomics approach, we identified H. pylori GroES as a dominant gastric cancer-related antigen, with a much higher seropositivity of gastric cancer samples compared to gastritis and duodenal ulcer. GroES seropositivity was more commonly associated with antral gastric cancer than with non-antral gastric cancer. In peripheral blood mononuclear cells, GroES enhanced the production of IL-8, IL-6, GM-CSF, IL-1β, TNF-α, cyclooxygenase-2, and PGE₂. When treated of gastric epithelial cells, with GroES let to expression of IL-8, cell growth and up-regulation of c-jun, c-fos, and cyclin D1, but caused downregulation of p27Kip1. We conclude that GroES of H. pylori is a novel gastric cancer-associated virulence factor and may contribute to gastric carcinogenesis via induction of inflammation and promotion of cell proliferation.

Protein Pre-fractionation Enhances Quantification of Differential Display of Protein Isoforms


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Distinguishing between isoforms is important in many proteomic applications. In this study we show that protein pre-fractionation can improve differential display of protein isoforms in the LC-MS workflow. Separation at the protein level not only reduces the complexity in the samples analyzed downstream with LC-MS but also gives the potential to put different protein isoforms in distinct fractions. By doing so, the chance to find statistically significant differences increases, since detection can be done not only on the peptides distinguishing the isoforms but on all peptides shared between protein isoforms. Standard protein samples as well as biological replicates of normal adjacent tissue and colon cancer samples were used to test this approach. Extracted and cleaned-up samples were separated on a strong anion exchange column (Mini QTM 4.6/50 PE) with an Ettan LC system. Buffer A consisted of 8 M Urea, 6% isopropanol and 20 mM Tris pH 8.5. Buffer B was Buffer A with 1 M NaCl added. Flow rate was 0.5 mL/min and the gradient was 0–40% in 30 min, thereafter 100% for 15 min. The eluate was fractionated into 250 μl fractions with fraction collector Frac-950. Fractions were digested and subsequently analyzed on an Ettan MDLC in connection to a Finnigan LTQ mass spectrometer. Data evaluation was done with DeCyder MS, TurboSEQUEST and GPM. Each analyzed fraction was found to contain 700–800 unique identified proteins (p < 0.01). Based on the intensity calculated in DeCyder MS, elution profiles corresponding to the protein level separation were plotted for of peptides shared among protein isoforms. This revealed that protein isoforms could be separated on the Mini Q column. The detection of a statistically significant difference for a low abundant isoform of a known cancer marker protein was also attributable to the protein pre-fractionation.
Membrane Proteomics of *S. cerevisiae* Vacuoles

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The vacuole of *S. cerevisiae* is the centre of many physiological processes in the cell. Vacuoles play a crucial role in pH- and osmo-regulation, ion homeostasis, degradation of proteins and organelles. Furthermore, it serves as a storage compartment for many kinds of substances and is an important component of an elaborate membrane system including the Golgi and ER. Vacuoles are surrounded by a single membrane (tonoplast) ensuring separation of the acidic vacuolar content from the cytoplasm. To allow an exchange of the vacuolar content with the cytoplasm and other organelles, transport processes across the tonoplast take place. A handful of membrane proteins has been identified or predicted to localize to the vacuolar membrane. In the light of the versatile physiological functions of vacuoles, more membrane proteins involved in transport are expected to be present. A proteomics approach was used for identification of novel proteins in the tonoplast of *S. cerevisiae*. Isolated vacuoles were converted into vacuolar membrane vesicles by osmotic shock and membranes were separated from the attached soluble and peripheral proteins by pH, salt stripping and an extensive EDTA-wash. To make an inventory of vacuolar membrane proteins, they were either separated by 1D-SDS followed by enzymatic digestion in-gel to generate peptides or digested directly in-liquid. In both cases peptides were separated by Liquid Chromatography and analyzed by tandem mass spectrometry. A major obstacle for the unambiguous assignment of the identified proteins to the vacuolar membrane is the presence of eventual contaminations and transient proteins. To investigate whether identified proteins represented random contaminant or were enrich along with known vacuolar resident proteins, a proteomics approach was applied. For this purpose, an isobaric tagging method (TMT) was used.

Proteome Analysis of Human Schizophrenic Brains


Max Planck Institute of Psychiatry, Munich, Germany; Inst. of Neurosciences, Inst. Psychiatry, USP, Sao Paulo, SP-Brazil; LAQUIP, Inst. of Biology, UNICAMP, Campinas, SP-Brazil; Central Institute of Mental Health, Mannheim, Germany

Schizophrenia (Scz) is a chronic, debilitating psychotic disorder that affects about one percent of the population. As a complex disease, Scz is likely to be a consequence of serial alterations of a number of genes that, together with environmental factors, will lead to the establishment of the illness.

Proteomic analysis of post-mortem Scz brains may lead to the identification of Scz-related proteins. We have collected the temporal lobe brain region of Scz and normal control brains and subjected them to comparative proteome analysis using Isotope Coded Protein Labeling. This method is based on a lysine-specific label with two differential isotope tags thus allowing protein comparison by mass spectrometry. Our results revealed an altered expression of proteins related with synaptic function, metabolic ways as well as cytoskeletal and cellular signaling. Our aim is to search for molecular targets that are Scz-related in order to identify markers for molecular diagnostic.

Heat Shock Protein 27 Proteome after Acid Pump Antagonist Treatment Contributed to the Prevention of NSAID-Induced Gastropathy


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Heat shock proteins (HSPs) are crucial for the maintenance of cell integrity during normal cell growth as well as during pathophysiological conditions, thereafter named as molecular chaperone. While functioning mainly as molecular chaperones through folding activities, heat-shock proteins also appear to be involved in diverse biological functions, such as apoptosis, carcinogenesis, and cytoprotection from cytotoxic and damaging stress. Biological activity of the HSPs is regulated elaborately by their cellular localization, phosphorylation status, oligomerization as well as expression level of the protein. In the stomach, induction of HSP27 in addition to HSP70 is revealed to play a very determining cytoprotective role in several gastric damages and rescue gastric epithelial cells from the injuries. However, relationship between its phosphorylation status and pathophysiological function in gastric diseases remains unknown. We therefore evaluated phosphorylation of HSP27 in NSAID-induced gastritis of rat using the proteomic analysis. We found that rat administrated indomethacin (40 mg/kg, ip) significantly decreased phosphorylation of HSP27 compared to control. While mice administrated revaprazan, novel acid pump antagonists prescribed for treatment of acid related gastric diseases including duodenal ulcer, prior to indomethacin exposure showed the restoration of its phosphorylation which is well correlated with prevention of the gastropathy. In conclusion, these results provide important novel insights into phosphorylation of HSP27 in response to NSAID-induced gastropathy and highlight the cytoprotective role of posttranslational modification of HSP27 in NSAID-induced gastric injury, leading to newer application of novel APA, revaprazan, for the treatment of NSAID-induced gastropathy.
Discovery of Proteomic Biomarkers Responsible for Alcohol-Induced Gastropathy

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Alcohol is one of major etiological factors for gastric mucosal injuries including gastritis and ulcer, which may progress to gastric cancer. Pathophysiologically, the alcohol-induced gastric mucosal damage can be mediated or modulated by various cellular molecules such as COX, LOX, cytokines, CYP2E1, TBX and oxygen derived free radicals, but very complex and complicated, by which exact either preventive or therapeutic interventions were not known yet. Even though the molecular works to reveal the underlying pathogenesis were done, including HSP, COX and MAPK, various gastric pathological conditions after alcohol administration necessitate further elucidations of responsible proteomes to explain the global pathogenesis. Here, we applied 2D-PAGE, image analysis, in-gel digestion, MALDI-TOF MS and protein identification for discriminating responsible proteomes of alcohol-induced gastropathy in rats. 20 spots were identified to be significantly decreased and 13 spots were significantly increased as follows: Increasing spots were apolipoprotein, mitochondrial aldehyde dehydrogenase, mammalian 2-cys peroxiredoxin, transgelin, titin-isoform etc., and decreasing spots were laminin receptor, calreticulin, ezrin, isocitrate dehydrogenase, actin β, eukaryotic translation elongation factor 2, aspartate transaminase etc. Two spots of glucose regulated protein were observed to be shifted to an acidic portion. Further validation of individual proteomes are now pending.

Age-related Changes in the Hippocampal Proteome of Cognitively Defined Rodents

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The etiology of the age-related decline in learning and memory is poorly understood. To determine those proteins that may contribute to these behavioral deficits, we behaviorally classified rodents using a hippocampally dependent task of learning and memory followed by a proteomics approach to assess alterations in specific proteins. Fisher 344xBN (F1) male rats were trained on a spatial reference memory water maze task (1 trial/day for 15 days) with a probe trial on day 16. The duration in the Annulus 40 over a 30s probe trial was used to separate animals into cognitive intact and cognitively impaired categories. One week later, animals were euthanized and hippocampus submitted for protein analysis. Protein was isolated from the whole hippocampus and the soluble fraction used for further analysis. Five animals each from the Young, Old-Intact, and Old-Impaired groups were randomly chosen and analyzed using a DIGE protocol (Freeman et al., 2005). 1,123 spots were matched across gels using DeCyder 6.0 software. The spot patterns demonstrated high resolution and a large number of spots across pH and molecular weight ranges. Initial statistical analyses of the results indicate that 43 proteins are differentially expressed between the three groups (p < 0.05) and that 26 proteins are differentially expressed between cognitively intact and cognitively impaired categories. One week later, animals were euthanized and hippocampus submitted for protein analysis. Protein was isolated from the whole hippocampus and the soluble fraction used for further analysis. Five animals each from the Young, Old-Intact, and Old-Impaired groups were randomly chosen and analyzed using a DIGE protocol (Freeman et al., 2005). 1,123 spots were matched across gels using DeCyder 6.0 software. The spot patterns demonstrated high resolution and a large number of spots across pH and molecular weight ranges. Initial statistical analyses of the results indicate that 43 proteins are differentially expressed between the three groups (p < 0.05) and that 26 proteins are differentially expressed between cognitively intact and cognitively impaired animals. Two spots of glucose regulated protein were observed to be shifted to an acidic portion. Further validation of individual proteomes are now pending.
Inhibitory Effect of Resveratrol on the Growth of Hepatic Cancer Cells


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Resveratrol exists in grapes and mulberry. It has potent chemopreventive effects against various tumors. We investigated effects of resveratrol in human hepatic cancer cells, Huh-7 and SK-HEP-1. The cells were treated with various concentrations of resveratrol for 1–5 days. We measured cell proliferation by MTT assay and checked morphological figures every 24 hours. The results of this study presented that the most effective concentration is 50 μM to 100 μM. The number of viable cells treated with 50 μM, 100 μM, and 250μM resveratrol were 70%, 50%, and 30% compared with control group, respectively. The lower concentration than 50 μM had no effect on cell death. Resveratrol inhibited the growth of cells in a time-dependent manner after 72 h. Resveratrol induced 40% cell death at treatment for 72 h to 96 h. As compared with cell number in control, that in treated with 50 μM to 100 μM group was increased about 10% by 72 h. Also we confirmed it by observing morphological figures. We had similarly results of resveratrol effect on Huh-7 and SK-HEP-1 cells. We have investigated differences of protein expression using 2DE in resveratrol treated SK-HEP-1 cells. Therefore, we suggest that resveratrol has inhibitory effect on human hepatic cancer cells. Further, we'll study the roles of resveratrol in hepatic cancer cells and find out what kinds of protein.

This work was done in the support of Korean Research Foundation.