16.1 Proteome Analysis of Hepatocytes Terminal Differentiation Using the MMH in Vitro Model
T. Alonzi, C. Mancone, L. Calvo, F. Ceradini, G. Fimia, M. Piacentini, and M. Tripodi
National Institute for Infectious Diseases, Rome, Italy

The liver maintains a balance between cell gain and cell loss. Cell proliferation and differentiation involve a gene expression modulation finely regulated by a complex network of stimuli such as growth factors stimulation and cell-cell contacts. The study of molecular mechanisms involved in this modulation is hampered by both the complexity of available in vivo models and the lack of proper in vitro models. In this work we attempt to assess if the untransformed immortalized MMH cell line could represent a suitable model for hepatocytes terminal differentiation studies. MMH (Met Murine Hepatocytes) cells were so far proven to retain several features peculiar of in vivo hepatocytes at phenotypic and functional levels. MMH cells, either in active proliferation (sub-confluent cells) or growth arrested in the G1 phase (confluent cells), where analysed for modification of mRNAs and proteins content. In particular, the proteome profile changes induced by cell-cell contact at membrane, cytosolic and nuclear levels were analysed by 2-DE and MALDI-TOF/TOF-MS.

We found that MMH cells upon cell-cell contacts, underwent to a complete cell cycle arrest and to a liver specific gene expression modulation. This modulation was strictly of cell-cell contacts, in fact cell cycle arrest mediated by the MEK1/2 specific inhibitor UO126 was not sufficient to induce liver specific gene expression. To better characterise the changes induced by cell-cell contacts, we performed proteomic analysis of cytosolic and nuclear extracts. Forty-five proteins were differentially present. Among them, 13 were more abundant or exclusively present in sub-confluent MMH cells. Interestingly, these proteins have been reported to play an important role in cellular proliferation such as Calumenin, Stathmin, Peroxiredoxin-2 and some members of the hsp70 family. Proteins more abundant in confluent MMH cells, are involved in hepatic functions, growth arrest mechanisms and cytoskeleton filaments assembly or stability. Moreover, the specific membrane bound proteome, revealed several post-translational modifications of proteins fundamental for epithelial cell polarity such as E-cadherin, Moesin and Ezrin. Our results indicated that MMH cells represent a suitable model to study the cell-cell contacts mediated hepatic terminal differentiation.

16.2 Impact of the NM23-M1 Metastasis Suppressor Gene Invalidation on Mouse Liver Proteome
M. Boissian1, A. Bruneel1,2, V. Labas3, J. Vinh3, S. Kyung Lim2, S. Arnaud-Dabernat4, J. Y. Daniel5, B. Baudin1,2, and M. L. Lacombe1

1INSERM 680, UPMC, Paris, France; 2Service de Biochimie A, Hopital Saint-Antoine, AP-HP, Paris, France; 3Laboratoire de Neurobiologie et Diversité Cellulaire, ESPCI, CNRS UMR 7637, Paris, France; 4Laboratoire de Biologie de la Différenciation et du Développement, EA DRED 3674, Université de Bordeaux 2, Bordeaux, France

We previously reported that the lack of the nm23-M1 gene markedly promotes metastases in an animal model of liver carcinogenesis (Boissian et al., J. Natl. Cancer Inst., 2005 in press). To better understand the mechanisms by which nm23-M1 is involved in metastatic process, we compared protein patterns of liver cytosol from nm23-M1 null mice (KO) with nm23-M1 wild-type mice (WT) using two-dimensional gel-electrophoresis (IPG pH 4–7), mass spectrometry (MALDI/TOF, LC-MS/MS) and Western blotting. We identified three proteins, i.e. phenylalanine hydroxylase (PAH), annexin IV and elongation factor 1 beta (EF-1 beta), whose levels were significantly decreased in liver cytosolic extracts from KO mice vs. WT mice. However, no concomitant decrease in corresponding mRNA levels, as assayed by real time PCR, were observed and no decrease of annexin IV and EF-1 beta protein levels was detected except for PAH when considering total liver lysates. Interestingly, annexin IV was increased in the hepatic nuclear fraction of KO mice as compared to WT mice suggesting that the lack of nm23-M1 plays a role in the nuclear to cytosol annexin IV equilibrium. Thus, nm23-M1 appears to control post-transcriptionally protein expression either at the synthesis and/or stability step or for the repartition between different sub-cellular compartments.

16.3 Protein Profilings in Mouse Liver Regeneration after Partial Hepatectomy Using iTRAQ Technology
S. Chen, Y. Chen, H. Hsieh, W. Wang, and T. Tseng
Industrial Technology Research Institute, Hsinchu, Taiwan

Limited understanding the complexity of regulatory mechanism of liver regeneration has brought the clinical interest to monitor the functional priorities after liver injury. Identification of biomarkers for liver regeneration has great potential in diagnostic and therapeutic applications. Protein profilings in mouse liver regeneration were present in this study by iTRAQ technology approach. Female C57B6 mice, aged 10–12 weeks, were performed 2/3 hepatectomy and were sacrificed spanning over a period of 120 h to obtain liver. Liver protein from each stage were denatured, cysteine blocked, tryptic digested and isoate labeled according to iTRAQ™ (Applied Biosystems, Foster City) protocol for semi-quantitative analysis. The pooled iTRAQ reagents labeled peptides were fractionated by strong cation exchange (SCX) chromatography. Each collected fraction was further analyzed by reversed-phased nanoLC-ESI-MS/MS (Applied Biosystems/MDS Sciei QSTAR pulsar i) and data were processed by Pro QUANT and Pro Group software for protein identification and calculated the overall quantitation ratios for each identified protein.

From combined strong cation exchange fractions, there were a total of 1946 peptides that associated to 362 proteins identified with 95% confidence or higher when search against IPI mouse database with all time points considered. Functional category illustrated metabolism-related proteins played significant roles. In those identified proteins, 43 proteins displayed at least 1.5-fold change in abundance when compared with the reference (no surgery). The identified proteins with significant change were also classified according to their functional properties and their kinetics of change after partial hepatectomy. Major groups of proteins were found to be functionally involved to detoxification and metabolism.
16.4 Changes in the Human Liver Proteome Induced by Transient Expression of Hepatitis Delta Virus RNA

M. Mendes1, D. Penque2, S. Mota1, A. Coelho3, and C. Cunha4

1Institute of Hygiene and Tropical Medicine, Lisbon, Portugal; 2Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal; 3Instituto de Tecnologia Química e Biológica, Oeiras, Portugal

The hepatitis delta virus (HDV) is a human pathogen that infects liver cells in association with the hepatitis B virus (HBV) and increases the severity of damage and the risk of fulminant disease. The HDV genome consists of a single stranded RNA molecule of about 1700 nucleotides, with a single open reading frame, that encodes a unique protein, the delta antigen (ΔAg). The RNA genome is replicated by RNA-directed RNA synthesis presumably involving cellular RNA polymerase II. Upon replication three RNA species accumulate: genomic, antigenomic, and 800 bp mRNA molecules. Due to its simplicity, HDV is highly dependent on host cellular factors and it is an excellent model of study for virus-host interactions.

Here, we aim to determine which changes arise in the cellular proteome as a consequence of transient expression of the HDV genome and antigenome alone. To achieve these goals we used 2D SDS PAGE for comparative studies. Human hepatoma Huh7 cells were transfected with constructs encoding antigenomic and genomic RNA, respectively (pDL481 and pDL542; kind gifts of Dr. John Taylor, Fox Chase Cancer Center, USA). Transfected Huh7 cells and the original cell line were analysed by 2D SDS PAGE, and displayed different expression patterns. The differentially expressed proteins were identified following the PMF strategy using spectra acquired on a MALDI-TOF type of mass spectrometer. We identified at least 35 proteins differentially expressed upon antigenomic HDV RNA expression, and 28 whose expression was altered during genomic HDV RNA replication. Most of the identified proteins are related to endoplasmic reticulum and other membrane functions as well as RNA processing.

16.5 Proteome Analysis of Detergent-resistant Membrane Rafts from Human Liver Carcinoma HUH7 Cells Harboring Hepatitis C Virus Subgenomic Replicon

C. Y. Fang1,3, Z. G. Yi2, F. Liu1,4, Q. P. Liu1,3, H. J. Lu1,3, Z. H. Yuan2,3, and P. Y. Yang1,3

1Department of Chemistry, Fudan University, 2Key Laboratory of Medical Molecular Virology, Shanghai Medical College, 3Institutes of Biomedical Sciences, Fudan University, and 4Chinese National Human Genome Center at Shanghai, Shanghai, China

Lipid rafts are domains of detergent-insoluble membrane which plays a critical role in many biological processes, such as regulators and organizing centers of signal transduction and membrane traffic pathways. It is reported that HCV RNA and non-structural (NS) proteins are present in detergent resistant membrane (DRM) structures, most likely a lipid-raft structure. To facilitate a better understanding of their biology and role in the HCV RNA replication, detergent resistant membrane rafts are isolated from human liver carcinoma Huh7 cells, because the Huh7 cells support the active RNA replication of a subgenomic HCV replicon. The fractions of detergent resistant membrane rafts are analyzed after being treated by 1% Nonidet P-40 at 4 °C and fractionated by differential centrifugation and discontinuous sucrose gradient centrifugation in this study. Totally 462 proteins including lipid raft markers are identified by 1D SDS-PAGE/LC/MS and 2DE/MALDI-TOFMS. All these proteins are involved mainly in signal transducer activity, kinase activity, GTPase activity, structural constituent of ribosome, transporter activity, and so on. A total of 217 (47%) proteins are classified as membrane proteins. In addition, our results reveal that 1D SDS-PAGE/LC/MS has advantages over 2DE/MALDI-TOF MS for the identification of membrane proteins. Of 217 membrane proteins, the 110 (50.7%) proteins are identified only by 1D SDS-PAGE/LC/MS and 55 (25.3%) proteins are analyzed only by 2DE/MALDI-TOF MS, with 52 (24%) overlapped proteins detected by the two methods.
16.6
SELDI-TOF-MS Profiling of Serum for Detection of Liver Cirrhosis and Hepatocellular Carcinoma in Chronic Hepatitis C
T. Göbel¹, S. Vorderwülbecke², K. Hauck¹, R. Schleucher¹, D. Häussinger¹, and A. Erhardt¹
¹Klinik für Gastroenterologie, Hepatologie und Infektiologie, Düsseldorf, Germany; ²Ciphergen Biosystems GmbH, Göttingen, Germany

The aim of the present study was to establish protein profiles using surface enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF-MS) for identification of liver cirrhosis and hepatocellular carcinoma (HCC) in patients with chronic hepatitis C. Serum samples of 40 patients with F1/F2 fibrosis, 44 patients with F4 fibrosis, 34 patients with HCC were applied to CM 10 cation exchange protein chips and analysed using the SELDI-TOF ProteinChip System (PBS-II; Ciphergen Biosystems) after anion-exchange fractionation. Fibrosis stage and HCC had been histologically confirmed in all patients. Data were analysed for protein patterns by multivariate statistical techniques and artificial neural networks. A 4 peptide/protein multimarker panel correctly identified HCCs with a sensitivity of 100% and specificity of 84% in the HCV-cirrhosis versus HCV-HCC training sample set. Sensitivity and specificity for HCC were 68% and 80% for an internal cross validated test sample set. Cirrhotic patients could be discriminated against patients with F1 or F2 fibrosis using a 5 peptide/protein multimarker pattern with a specificity of 100% and a sensitivity of 85%. Whereas the internal cross validation test set displayed sensitivity and specificity for liver cirrhosis of 80% and 67%, respectively. SELDI-TOF-MS technology combined with protein pattern analysis seems a valuable approach for the identification of liver cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis C. Due to the suitability of the SELDI-TOF-MS/ProteinChip technology for mass application it might become a tool for screening HCV patients for cirrhosis and HCC.

16.7
Changes in Hepatic Proteome during Fibrogenesis; DIGE (Difference in Gel Electrophoresis) Reveals New Proteins Like Selenium Binding Protein 2 in the Context of Fibrosis
C. Henkel, M. Roderfeld, M. Berres, S. Wagner, K. Stuehler, S. Matern, and E. Roeb
University Hospital RWTH Aachen, Aachen, Germany

Liver fibrosis, which is an outcome of persistent hepatic inflammation, if left unmanaged has serious long-term consequences for the patient. We investigated the changes in hepatic proteome during fibrogenesis to get an idea of protein changes in this context. Hepatic fibrosis was induced by intraperitoneal treatment with CCl₄ and the protein pattern of injured liver was compared to healthy liver of inbred mice. Changes in protein pattern could provide information about proteins participating in the progression of liver fibrosis. Furthermore these proteins might be fibrotic markers with clinical relevance. We used the innovative DIGE (Difference In Gel Electrophoresis) technique to analyse the expression levels of proteins in the mouse liver proteome. We detected interesting candidate proteins like selenium binding protein 2 (sbp2), carbonic anhydrase 3 and glutathion-S-transferase which showed modified expression patterns during hepatic fibrogenesis. We focused our interest on sbp2 because it showed the strongest significant (down) regulation of all proteins we found. It is known that oxidative stress is a direct stimulus for fibrosis and we postulate a role of sbp2 in this process. We confirmed our results on protein level by real time PCR of the corresponding mRNA. In conclusion DIGE offers a powerful tool in proteome analysis to discover new promising protein markers for diagnosis of chronic hepatic diseases.

16.8
Disease Stage-specific Protein/Peptide in HCV-related Liver Cancer
T. Katagiri¹, K. Shiraki², H. Fujimoto³, Y. Takeda⁴, E. Yamaguchi⁵, K. Meno⁵, and K. Uchida⁵
¹Molecular and Clinical Bioinformatics, Inc., Tsukuba, Japan; ²The 1st Internal Medicine, Mie University Hospital, Tsu, Japan; ³Shimadzu Corporation, Kyoto, Japan; ⁴NTT Comware Corporation, Tokyo, Japan; ⁵Graduate School of Comprehensive Human Science, University of Tsukuba, Tsukuba, Japan

Hepatocellular carcinoma (HCC) is one of the most fatal cancers with a high incidence in many countries. Hepatitis C virus (HCV) is the most clearly established risk factor for HCC. HCV carriers proceed to chronic hepatitis (CH), to liver cirrhosis (LC), and to HCC after incubation periods of 15, 25, and 30 years in average, respectively. Biomarkers for recognition of these processes are necessary for correct treatment of the patients. Especially, biomarker for LC is important since more than 70% of HCV-induced LC is destined to HCC. In this study, we tried to find specific biomarker protein or peptide for these liver diseases in the serum from the 20 patients. For this aim, we performed differential proteomics between patients and healthy persons by three means such as 2D-HPLC-MALDI-TOF/MS, SELDI-TOF/MS and 2D-PAGE. Our unique 2D-HPLC-MALDI-TOF/MS identified two peptides appeared specifically in CH, two peptides in HCC and four in LC, and to HCC, while one disappeared specifically in LC and three in HCC. SELDI-TOF/MS demonstrated seven peptides showing high intensity in CH and four ones in HCC, while ten peptides in these patients with lowered intensity as compared to normal controls. 2D-PAGE analyses exhibited one serum protein specifically appeared in these liver diseases and four serum proteins of normal controls disappeared in most of LC patients but not in CH. Combination of the two of these proteins demonstrated 90% sensitivity and 96% specificity in discriminating LC patients from CH ones. Our data together indicate that serum proteins and peptides in combination will become better diagnostic markers and lead to correct treatment of liver diseases.
16.9
Proteomics as a Part of Systems Biology
Digging for Membrane Proteins
E. Langenfeld1, G. Redlich1, T. Schultenborg1, U. Zanger2, G. Vacun3, K. Marcus1, and H. Meyer1
1Medizinisches Proteom Center, Bochum, Germany; 2Margarete Fischer Bosch Institut für Klinische Pharmakologie, Stuttgart, Germany; 3Institut für Bioverfahrenstechnik, Stuttgart, Germany

Systems biology is an approach in biology, that tries to integrate knowledge arising from different disciplines of research, such as RNA-profiling, metabolism studies and proteomics. The goal is to create a mathematical model that helps understanding the systems behaviour under various conditions. Since translation of proteins does not correlate with mRNA expression, it is crucial not only to analyze a systems transcriptome but also the proteome. In our network we want to create a model for the metabolism of the human hepatocyte. One important function of the liver is the metabolism of xenobiotics. These are degraded and modified by a number of cytochrome P450 monoxygenases, transferases of hydrophilic groups and various transporters, forming a network that is regulated by interacting nuclear receptors. The task of our project is therefore to adapt sample preparation and separation of membrane proteins of primary rat and human hepatocytes in a way that allows us to study as many of these proteins as possible. We pursue a twofold strategy combining gel-based separation techniques with a chromatographic approach. In this work we present two-dimensional gel electrophoresis with the cationic detergent CTAB and first steps of a chromatographic approach in the analysis of membrane proteins. The gel-based analysis showed first promising results in the separation and identification of drug-metabolizing enzymes of the smooth ER. For the chromatographic approach microscale solution IEF (μsol-IEF) should be combined with ion chromatography. Albeit the proteins being properly fractionated in μsol-IEF, some restrictions were observed, which were due to the system’s design. The system is suitable for protein purification, however for differential analysis further optimization is required.

16.10
Molecular Basis of Cell Detachment and Biliary Tract Pathology Caused by the Fungal Toxin Sporidesmin
G. Lindsay, M. Johnson, A. Bala, K. Hellier, and B. Jordan
Victoria University of Wellington, Wellington, New Zealand

The fungal toxin sporidesmin causes necrotising inflammation of bile ducts followed by secondary hepatocellular damage. We have examined the effects of this toxin on the liver HepG2 cell line and on primary cultures of biliary epithelial cells. Exposure to the toxin caused rapid loss of cell-cell contact and detachment of cells from tissue culture plastic. These changes were preceded by disruption of actin microfilament cables. Prior exposure of cells to the microfilament stabilizing compound Jasplakinolide protected against cell detachment indicating that loss of cell contact was secondary to microfilament disruption. Using model recombinant proteins we have previously shown that sporidesmin selectively modifies specific sulphydryl residues of a small number of protein targets. We have therefore commenced a 2DE analysis of the effect of sporidesmin on cellular proteins including a search for the targets of sporidesmin action. This work is of importance for understanding the natural toxicity of sporidesmin and structurally related sulfur-bridged dioxopiperazine toxins (Jordan & Cordiner Trends in Pharmacological Sciences 1987, 8, 144–149).

16.11
Human Liver Proteome Changes Induced by the Hepatitis Delta Virus
S. Mota1, D. Penquè2, M. Mendes1, A. Coelho3, and C. Cunha1
1Institute of Hygiene and Tropical Medicine, Lisbon, Portugal; 2Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal; 3Instituto de Tecnologia Química e Biológica, Oeiras, Portugal

The hepatitis delta virus (HDV) is the smallest human virus and the only satellite virus known so far. For the production of infectious particles the HDV requires the presence of the hepatitis B virus (HBV) surface proteins. Hepatitis delta patients develop more severe symptoms than patients infected only by the HBV. Due to its simplicity this virus is highly dependent on host cell factors, and represents an excellent model for studying virus-cell interactions.

In this work we aim to understand the cellular changes that occur during the replication of the HDV. We analysed the cellular extracts of two different cell lines. The human hepatocellular carcinoma cell line (Huh7) was compared with the correspondent HDV cDNA stably transfected cell line (Huh7-D12) that constitutively expresses the virus ribonucleoproteins. The comparative studies were performed using two-dimensional polyacrylamide gel electrophoresis (2D SDS-PAGE). Spot detection and matching was performed using the ImageMaster software where as statistical analysis was performed with the Ludesi 2D Interpreter software. The differentially expressed proteins were identified following the PMF strategy using spectra acquired on a MALDI-TOF type of mass spectrometer.

We detected expression differences and identified at least 20 proteins. Some of the downregulated proteins are also downregulated by the Hepatitis C non-structural proteins NS4B and by the Rous Sarcoma Virus. Some of the upregulated proteins have previously been reported to interact with the HBV large surface antigen and increase viral production and proteins synthesis in the mouse hepatitis virus.

16.12
Identification of Cellular Factors Associated with the 3'-Nontranslated Region of the HCV Genome by RNA Affinity Capture and Mass Spectrometry
V. Pandey, D. Harris, Z. Zhang, and S. Ganguly
UMDNJ-New Jersey Medical School, Newark, New Jersey, USA

Chronic infection by Hepatitis C virus (HCV) is the leading cause of severe hepatitis which often develops into liver cirrhosis and hepatocellular carcinoma. The molecular mechanisms underlying HCV replication and pathogenesis are poorly understood. Similarly, the role(s) of host factors in the replication of HCV remain undefined. Based on our knowledge of other RNA viruses, it is likely that a number of cellular factors may be involved in facilitating HCV replication. It has been demonstrated that elements within the 3' nontranslated region (3'NTR) of the (+) strand HCV genome are essential for initiation of (+) strand synthesis. The RNA signals within the highly conserved 3' NTR may be the site for recruiting cellular factors which mediate virus replication/pathogenesis. However, the identities of putative cellular factors interacting with these RNA signals have been unknown. In this report, we demonstrate that an RNA affinity capture system developed in our lab used in conjunction with LC/MS/MS mass spectrometry has allowed us to positively identify more than 70 cellular proteins that specifically interact with the 3'NTR (+) of HCV. The binding of these cellular proteins could not be competed out by a 10-fold excess of nonspecific competitor RNA. With few exceptions, all of the identified cellular proteins are RNA binding proteins whose reported cellular functions provide unique insights into host cell-virus interactions and possible mechanisms influencing HCV replication and HCV-associated pathogenesis.
16.13
Strategy Optimization for the Constructing of Chinese Human Liver Proteome Expression Profile
X. Qian, Y. Zhu, Y. Zhang, S. Wu, Y. Gong, F. Zhong, and F. He
Beijing Institute of Radiation Medicine, Beijing, China

To construct the Chinese human liver proteome expression profile, a series of critical experiments was performed in the laboratories to evaluate and develop standardized procedures for sample collection and preparation, organelle preparation and characterization, protein fractionation and analysis and data process and submission.

Among these, a special experiment was designed with eighteen of standard commercialized proteins to evaluate the strategies to be utilized by different laboratories for fractionation and analysis of human liver proteins. The proteins listed were analyzed firstly both in intact protein level and in peptide level by MALDI-TOF-TOF-MS and nano-LC-ESI-MS-MS separately. Then two sets of sample, one for gel-based strategy and another for shotgun were prepared separately with different concentration. To database searching, an integrated database was developed based on the categories of the proteins. The experiment was performed and the data were processed and analyzed. The preliminary results showed that different strategies have different power to detect and identify the proteins in different concentration level. Among the techniques used in the experiment, the strategy of SDS-PAGE combined with in gel digestion and nano-LC-ESI-MS-MS showed the highest detection rate (the ratio of detected standard proteins to eighteen) in all strategies. 2DE-based method identified a fewer proteins than SDS-PAGE method, but its correct rate (the ratio of detected standard protein groups to detected all protein groups) was higher. Because the molecular weight of the detected proteins was relatively consistent with the detective frequency of the protein, the proteins were identified by the MALDI-TOF/TOF MS analysis. The throughput, accuracy, sensitivity and resolution of protein expression. The different strategies and methods supplied complementary information to the experiment, the strategy of SDS-PAGE combined with in gel digestion and nano-LC-ESI-MS-MS showed the highest detection rate (the ratio of detected standard proteins to eighteen) in all strategies. 2DE-based method identified a fewer proteins than SDS-PAGE method, but its correct rate (the ratio of detected standard protein groups to detected all protein groups) was higher. Because the molecular weight of the detected proteins was relatively consistent with the detective frequency of the protein, a protein fractionation step based on the molecular weight would be effective in complexity sample analysis.

16.14
Sequential Serum Proteomic Profiles in Hepatitis B Surface Antigen Carriers with an Acute Exacerbation
1Chang Gung Memorial Hospital, Taipei and 2Academia Sinica, Taipei, Taiwan

Acute exacerbation (AE) of liver inflammation occurs during the course of chronic hepatitis B virus (HBV) infection, which is an immune response aims to terminate HBV replication. However, the AE may result in hepatic decompensation or even death. The mechanisms that trigger the immune clearance response and parameters that may predict the outcome of the AE remain to be elucidated. Recent advances in the proteomic technologies open a new window to examine the changes in the protein expression for human disease progression. Seven hepatitis B surface antigen positive patients with AE were enrolled in this study. Sequential sera in pre-, peri-, and post-AE stages were examined with two-dimensional gel electrophoresis. The Image Master Software determined intensities of spots and the proteins were identified by the MALDI-TOF/TOF MS analysis. The complement C4 and ficolin levels did not change in the peri-AE period, whereas; complement H, transthyretin, haptoglobin a2 and b chains levels were dropped significantly at the peak of AE. a-1-antitrypsin was found to be elevated at pre-AE stage in some patients. Serum apolipoprotein did not drop in AE; rather it was rising progressively up to the post-AE stage. The drop of complement H level suggests a role of complement alternative pathway in AE. Carefully analyze protein profile patterns may add new information in host inflammatory response, which may be of help in understanding the pathogenesis, predicting the outcome and approaching to multi-biomarker diagnostics in chronic liver diseases and hepatocellular carcinoma.

16.15
Systematic Analysis for Liver and Liver Cancer Proteome; From Laboratory to Bedsides
R. Zeng, L. Chen, Y. Tan, J. Wu, H. Wang, and Q. Xia
1Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai and 2Eastern Hepatobiliary Surgery Hospital, Shanghai, China

It is well-known that one of the most important features of proteome is that proteins are expressed with an extremely dynamic mode, which related to protein function, interaction and activity. Therefore, the qualitative and quantitative analyses of proteome are the critical steps to investigate the correlations of proteins and disease or physiological process. We used novel multiple dimensional liquid chromatography based on pH step elution to achieve more convenient and reliable qualitative analysis for liver proteome only using one-dimensional liquid chromatography system. Furthermore, based on tissue or cell samples, we applied in-vitro or in-vivo stable isotope to label proteins followed by gel methods and LC-MS/MS identification. We used prefractioantation assay to enrich low-abundant proteins before obtain their expression level by mass spectrometry. On the other hand, fluorescent dye was used to label proteins before two-dimensional electrophoresis and protein differential expression was measured. The different strategies and methods supplied complementary information of protein expression. The throughput, accuracy, sensitivity and resolution were compared among these methods. About 1000 differential proteins were obtained by combining the above strategies. Further analysis includes clinical immunohistotical, protein fluorescent location and protein interaction were carried out to find out protein dynamics in the HCC carcinogenesis. This systematic study on liver and liver cancer proteome may be just the beginning but already shed some lights on the mechanism of HCC carcinogenesis and progression.
Search for HBsAg-associated Proteins in the Livers of HBV Transgenic Mice with Proteomic Analysis

C. Zhao¹, C. Y. Fang²,³, P. Y. Yang²,³, and Y. M. Wen¹

¹Key Laboratory of Medical Molecular Virology, ²Department of Chemistry, and ³Institutes of Biomedical Sciences, Fudan University, Shanghai, China

The mechanisms for the persistence of HBsAg in chronic HBV infection have not been fully explored. To analyze the possible metabolic pathways involved in HBsAg persistence, we employed a proteomic approach to compare the expression profiles in the livers of HBsAg⁺(HBsAg positive) transgenic mice and their control siblings which were HBsAg⁻(HBsAg negative), established by our collaboration with the Shanghai Center of Model Organisms. In this study, pooled livers of three HBsAg⁺ transgenic mice and of three HBsAg⁻ counterparts were globally examined by 2D-gel electrophoresis with three times repeats followed by matching of gels grouped. Differentially expressed spots with the density ratio of HBsAg⁺/HBsAg⁻ or HBsAg⁻/HBsAg⁺ above 1.5 were identified by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS), followed by database searching. Totally, 61 proteins were found increased in HBsAg⁺ mice liver while 34 were decreased. When analyzed, these proteins were mainly categorized into metabolism, including carbohydrate, amino acid and fatty acid, and protein modification, signal transduction and others. Some key enzymes of metabolism such as OAT (ornithine aminotransferase), Fdps (farnesyl diphosphate synthetase), GPD1 (glycerol phosphate dehydrogenase 1), involved in the physiological process maintaining the cellular and molecular functions were changed. Among these, the ornithine transcarbamylase (OTC) decreased to 0.383 of the control mice, and was predicted to reduce the synthesis of urea. This might lead to accumulation of ammonia in blood and related to liver and brain injury. While analyzing the results we plan to use biochemical assay methods to study whether these changes in metabolism did occur in the transgenic mice and the possible significance of these changes.