8.1

Oxidant and Antioxidant Status in the Hypo- Hyperthyroid Patients Before and After Treatment

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Free oxygen radicals play an important role in the etiopathogenesis of many diseases. In this study, we examined the relationship of oxidant and antioxidant status in the hypothyroid and hyperthyroid patients before and after treatment. This study composed of 21 hyperthyroid,18 hypothyroid patients and 32 control subjects. The level of hormone, serum MDA, Zn, Cu, Mg, erythrocyte antioxidant enzymes and the level of plasma vitamins A, E were determined in the blood samples before and after treatment. It was determined hyperthyreotics patients showed a significant increment in MDA while hypothyreotics patients showed no significant change in MDA when compared to those of control subjects according to before treatment. It was also observed that both patient groups showed an increment in erythrocyte catalase activities and a significant decrement in GSH-Px and a slight decrement in SOD after treatment versus pretreatment value. Compared to pretreatment values MDA levels were markedly lower in both groups ($p < 0.015$ for hyperthyroitic and $p < 0.041$ for hypothyrotic) while no significant changes were determined in erytrocyte CAT, GSH-Px, and SOD levels. Before treatment, It was detected that vitamin E levels were lower in both groups than control subjects. However, vitamin A levels were almost the same in both groups versus the control subjects. After treatment, we also observed that vitamin E level in both groups demonstrated an increment. There was no change in the level of Zn and Cu in both groups after and before treatment terms however, Mg level compared with before treatment It was found that Mg level of patients were higher than control subjects whereas the level of Mg shown a decrease after treatment. These results suggest that supplementation diet with antioxidant micronutrients might provide a protection against cellular damage caused by thyroid pathogenesis in both hypothyroid and hyperthyroid patients and it was thought that this antioxidant micronutrient supplementation might positively influence the life quality.

8.2

Proteomics of the Early Secretory Pathway of Rat Liver with an Emphasis on Detoxification Enzymes

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Isolation of highly enriched sub cellular fractions of rough and smooth endoplasmic reticulum, Golgi apparatus and COPI vesicles from rat liver homogenates led to the characterization of 994, 1208, 838, and 600 different proteins in the respective compartments. For the smooth endoplasmic reticulum, the most abundant proteins represented those involved in bile acid formation and drug detoxification. Tandem mass spectrometry characterized over forty different cytochrome P450 members, whose relative abundances were estimated. Ten different glucuronosyl transferases were characterized, and 10 different carboxyl esterases were found including those only predicted previously as potential open reading frames. The oxidation of cholesterol and its derivatives by the cytochrome P450 detoxification system is coupled to electron transport via NADPH cytochrome P450 reductase whose relative abundance was also deduced. This electron transport system also interacts with a lipid desaturating machine consisting of NADH cytochrome B5 reductase and cytochrome B5 whose abundances were also deduced. These molecular machines are part of an even larger system wherein fatty acids and cholesterol are biosynthesized and oxidized by enzymes associated with the endoplasmic reticulum. These proteomics studies define a compendium of the highly abundant molecular machines of the liver endoplasmic reticulum involved in lipid and cholesterol biosynthesis, bile acid formation, steroid inactivation and clearance, and drug detoxification.

8.3

Proteome Database: The Fetal Liver Analysis

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Human fetal liver in 4–6 months of gestation performs physiological functions of normal liver as well as embryonic hematopoiesis and immune development. Therefore, the unique characteristics of the fetal liver at this stage are worthy of investigation. Gene expression profiling of HFL22w has been constructed, a further complementary study in proteome give a comprehensive understanding of the unique functional characteristics of HFL22w. And also provide a global view on information such as proteins expression, post-translational modifications.

The necessities for large scale of proteome research are separation techniques with high resolution and proteins identification techniques with high throughput and high sensitivity. Firstly narrow range and ultra-zoom 2-DE separation of fetal liver proteins were optimized. By the 2-DE techniques, a high resolution 2-DE map of fetal liver total proteins with 5487 protein spots detected was presented. Secondly, techniques for high throughput identification of in-gel protein from 2-DE gels and SDS-PAGE gels were established and optimized.

Using sub-proteome technique combined with 2-DE, SDS-PAGE and IPG IEF separation and multi-stage mass spectrometers, we carried out the proteome profiling of fetal liver total proteins as well as the fetal liver cytoplasmic proteins. 864 proteins comprised of 687 unique proteins and 177 clusters were measured from total proteins. While more proteins were detected from cytoplasmic, including 1480 unique proteins and 348 protein clusters. Functional categories and relative abundance analysis of fetal liver proteins were performed. A number of proteins specifically related with normal liver, liver cancer, hematopoiesis and development were inferred after comparisons between fetal liver proteome database and nine other proteome databases.

8.4

Identification of Biomarkers for Peroxisome Proliferation and Its Associated Hepatocarcinomas by Proteomics Approaches

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Peroxisome proliferators constitute a group of chemicals with many applications in healthcare industry. Administration of peroxisome proliferators to rodents, results in marked hepatomegaly that is characterized by increased expression of enzymes involved in fatty acid metabolism, proliferation of peroxisomes in hepatic parenchymal cells, and increased liver cell proliferation during early stages. Chronic exposure to peroxisome proliferators leads to the development of hepatocellular carcinomas in rats and mice due to nongenotoxic mechanisms. The effects of peroxisome proliferators in rodents are mediated by peroxisome proliferator-activated receptor α (PPARα), a member of the group of transcription factors that regulate the expression of genes involved in lipid metabolism and adipocyte differentiation. In an effort to identify biomarkers for peroxisome proliferation and subsequent hepatocarcinoma, we used fluorescence two-dimensional differential in-gel electrophoresis (2-D DIGE) to obtain comparative liver protein profiles of Acyl-CoA Oxidase deficient ($AOX^{-/-}$) and Wy-14,643-treated mice. Differentially expressed protein spots were selected and identified by liquid chromatography coupled tandem mass spectrometry. A set of proteins previously unassociated with PPARα-regulation was identified. The upregulated proteins, including acetly-CoA acetyltransferase, farnesyl pyrophosphate synthase, and carnitine O-octanoyltransferase, are involved in fatty acid metabolism, whereas the down regulated proteins, such as ketohexokinase, formiminotransferase-cyclodeaminase, fructose-bisphosphatase aldolase B, sarcosine dehydrogenase, and cysteine sulfinic acid decarboxylase, are related to carbohydrate and amino acid metabolism. Among stress response and xenobiotic metabolism proteins, selenium-binding protein 2, and catalase showed, respectively, a dramatic ~18-fold decrease and a modest ~6-fold increase. In addition, glycine N-methyltransferase, pyrophosphate phosphohydrolase, and protein phosphatase 1D were down regulated with PPARα activation. These observations establish predictable proteomic profiles reflecting a common pattern of differential protein expression in livers with PPARα activation, either by natural ligands as in $AOX^{-/-}$ mice, or by synthetic ligands as in wild type mice treated with peroxisome proliferators. A subset of up- and down-regulated proteins are selected and proposed as potential biomarkers for peroxisome proliferation and its associated hepatocarcinomas (*Mol. Cell. Biol.* **24,** 6288–6297).

8.5

Proteomic Analysis of Hepatocarcinoma in *Mat1a* Ko Mice. Molecular Pathogenesis and Biomarkers

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Hepatocellular carcinoma (HCC) is the fifth most common neoplasm with more than 500.000 new cases diagnosed yearly. Major risk factors of HCC are currently known, including hepatitis B and C viruses, ingestion of aflatoxin contaminated food and alcohol abuse. However , the molecular pathogenesis of HCC is yet poorly understood. Recent studies show that S-adenosylmethionine (AdoMet) helps maintain normal liver function as chronic hepatic deficiency results in spontaneous development of steatohepatitis (NASH) and HCC. In this work we use a knockout mice deficient in hepatic AdoMet synthesis (*MAT1A*-/-) to study the proteome of the liver during the development of HCC. The analysis of tumor nodules from different mice revealed that more than 10% ($p < 0.05$) of the proteins changed their expression pattern while the variability within any other experimental group was lower than 1% ($p < 0.01$). Our results suggest that proteome heterogeneity not only derives from a stochastic process associated to tumor growth but also that mouse-specific factors might account for the unstability by imposing a selective pressure on preneoplastic foci. We have identified 170 proteins differentially expressed in MAT1A-/- mice HCC, and 21 are common to more than 50% of the analyzed tumors. Among them, changes on heat shock cognate 71, glutamine synthase, and glutathione S transferase μ isoform have been found in human HCC. We have also identified a specific isoform of apolipoprotein A1 (ApoA1) in the serum of MAT1A-/- mice much earlier than any histological manifestation of HCC. This acidic isoform of ApoA1 was also detected in HBV patients that developed HCC. ESI/MS/MS analysis revealed that this form is a highly oxidized ApoA1 in methionine and tryptophan residues. In summary, our results provide a global picture of proteome alterations associated to HCC development with functional implications and a potential value in biomarkers identification.

8.6

Study on Normal Chinese Human Liver Proteome Profile by 2D Gel Electrophoresis and MALDI-TOF-TOF-MS

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Currently, one of the most popular application of proteomics is in the area of protein expression profile of cell lines or tissues. This research was initiated with the purpose of separating and identifying more proteins of normal human liver tissue as much as possible. The powerful techniques used in this study is high-resolution two dimensional gel electrophoresis (2DE) combined with matrix assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF-MS). The liver samples were divided into two groups: one for total protein extraction and the other for protein sequential extraction by using BioRad sequential extraction kit. The protein separation was performed on pH 3–10 nonlinear or pH 4–7 linear IPG-strips and a 12.5% precast SDS-gel, stained with CBB, CCB or Ag. All protein spots were picked and digested with 3 μl 12.5 ng/μl trypsin in 20 mmol/l NH_4HCO_3 for 3 hours automatically. The tryptic peptides were extracted by 50% CAN-0.1% TFA. The peptide solutions were dried under N_2 followed by re-dissolving in 0.7 μl solution containing 4 mg/ml CHCA-2 mg/ml citric acid (ammonium salt)-50% ACN-0.1% TFA solution, and then spotted onto the MALDI target of the 4700 MS instrument. The detectable peptide masses were searched for matches in IPI database and the proteins were identified according to PMF combined with MS/MS. The protein population were found more than 1700 for the total extraction, and 1500 protein spots could be detected in E1 and E2 extraction respectively when the gels were visible by silver staining method, and more than 250 in E 3 part. The MS identification now is under process.

8.7

Identify Hepatocellular Carcinoma Associated Autoantibodies by SERPA Analysis

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Purpose: To screen hepatocellular carcinoma (HCC) associated autoantibodies in serum as diagnosis or prognosis biomarkers by serologic proteome analysis (SERPA), a newly developed powerful approach which combines conventional proteome analysis with serological screening.

Methods: Total proteins extracted from both tumor and non-tumor counterpart tissues were separated by two-dimensional electrophoresis (2-DE) and the separated proteins were then transferred onto PVDF membranes which were subsequently incubated with sera from HCC patients or healthy volunteers. Detection of immunoreactive proteins was performed with a secondary antibody directed against human IgA+G+M and visualized by autoradiography. By image analysis and 4700 Proteomics Analyzer (MALDI-TOF-TOF-MS), the different immunoreactive proteins were screened out and identified. The further validation of these discovered proteins was carried out by tissue array.

Results: 2-DE and corresponding western blot imaging maps of good quality and reproducibility were established. The average number of immunoreactive spots detected using sera from HCC patients or healthy volunteers were 64.63 $\pm$ 32.1 and 14.38 $\pm$ 7.5 respectively. By identification, those proteins that induced the generation of HCC associated autoantibodies could be classified as cytoskeleton proteins (CK19, CK20 and vimentin etc), nuclear proteins (nucleolin and laminA/C), molecular chaperones (gp96 and HSC70) and metabolic enzymes (thioredoxin peroxidase, aldose reductase etc). The result from tissue array indicated that CK20, gp96 and HSC70 were expressed only in HCC tissue and nucleolin, vimentin, laminA/C, thioredoxin peroxidase were over-expressed significantly in HCC tissue compared with normal control.

Conclusions: The carcinogenesis and development of HCC can result in the alteration of protein expression, which may induce the generation of tumor associated autoantibodies in serum from HCC patients. These HCC associated autoantibodies can be combined into a serum biomarker panel which could be used in the diagnosis or prognosis of HCC.

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8.8

Serum Proteome Analysis of Primary Hepatocellular Carcinoma by Two-dimensional Gel Electrophoresis and Matrix-assisted Laser Desorption-Ionization Time of Flight Mass Spectrometry

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Hepatocellular carcinoma (HCC) is the fourth most common cancer worldwide. Its incidence rate, however, has been increasing over the last two decades of the 20th century. Alpha-fetoprotein is the most widely used tumor marker, but has poor diagnostic accuracy. Using proteomics analysis, new candidate tumor markers will be identified. Analyzing the serum proteome of the patients suffering from primary hepatocellular carcinoma (PHCC) and healthy donors by proteomics techniques to search novel or more sensitive tumor markers of hepatocellular carcinoma for diagnosis. The serum proteome of the patients from primary hepatocellular carcinoma and healthy donors were separated and identified using two-dimensional electrophoresis (2-DE). Immobilized pH gradient isoelectric focusing (IPGIEF) electrophoresis was run as the first dimensional electrophoresis, and then horizontal SDS-PAGE as the second electrophoresis. After Coomassie blue staining, images were captured by image-scanner and then the images were edited and matched using Imagemaster 2-DE analysis software. The differentially expressed proteins were analyzed by peptide mass fingerprint based on matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF-MS) and SWISS-PROT or BLAST nr database searching. We also advanced some critical procedures of serum proteome 2-DE. Trying different loading quantity, procedure of isoelectric focusing electrophoresis, time of equilibrium, density of SDS-PAGE gel and other critical procedures of two-dimensional electrophoresis, the images were analyzed successfully and good reproducibility were obtained. Analyzing by Imagemaster2DE software, thirty-three proteins were found differentially expressed in sera from hepatocellular carcinoma patients and healthy donors. Six proteins were down-regulated in PHCC, five proteins were up-regulated in PHCC, seventeen proteins were found especially in healthy controls and five proteins were found especially in PHCC. After analyzing by MALDI-TOF-MS, five differentially expressed proteins were identified. Albumin 'Serotransferrin' CD5 antigen-like precursor (IgM-associated peptide) were down-regulated in PHCC, and Zinc-alpha-2-glycoprotein and Ig gamma-1 chain C region were up-regulated in PHCC. Using immobilized pH gradient two-dimensional polyacrylamide gel electrophoresis (2-DE), good reproducibility and images could be obtained to separate and identify the proteome in serum. Thirty-three proteins that were found differentially expressed in PHCC and healthy donors provided useful information for screening diagnostic tumor markers of human PHCC.

8.9

Proteomic Analysis of Differential-expressed Proteins in Rat Liver After 2/3 Hepatectomy

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The two-thirds partial hepatectomy (PHx) model of rat liver provides an effective way to study the transition and regulation of hepatocytes from quiescent phase to proliferating phase. Although the gene-expression pattern has come under intense scrutiny, a differential proteomic study could better help to reveal the mechanism how the process is initiated and regulated. First, a time point of 7 hr after 2/3 PHx was determined to perform the differential proteomic analysis between the quiescent phase and the priming phase (in which hepatocytes "prepared" to proliferate) based on a time point screen by 1-D gel analysis. The proteomic changes were then analyzed on three groups: normal hepatocytes, 7 hr after 2/3 PHx, and the sham-operation control by two-dimensional gel electrophoresis. 18 down-regulated and 23 up-regulated spots were recognized by using the 2D analysis software ImageMaster with the criterion of at least two-fold expression change. Among them, 28 proteins were identified by MALDI-TOF-MS. It was notified that some proteins were identified from more than one spots on the 2D gel, suggesting those proteins might be degradated in different ways in quiescent phase and priming phase cells. Function analysis of identified proteins using protein database showed that these proteins may be involved in the metabolism process, protein transporting and folding, Ca^{2+} modulation, cell skeleton construction *et al.*

8.10

Comprehensive Proteomic Analysis of Plasma Membrane from Human Fetal Liver During 16–24 Weeks of Gestation

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The plasma membrane (PM) constitutes the interface between eukaryotic cells and their external environment. Consequently the functions of proteins embedded in this membrane include cell/cell and cell/extracellular matrix recognition, the reception and transduction of extracellular signals and the transport of solutes and water molecules into and out of the cell. Especially in human fetal liver (HFL), this function of plasma membrane is very important for liver development, cell differentiation and hematopoietic of fetal liver. Moreover, the plasma membrane has been extensively targeted for drug design; plasma membrane proteins account for 70% of all known drug targets.

PM of HFL was isolated by differential centrifugation and sucrose dense centrifugation. Due to unsatisfactory separation of integral membrane proteins from higher eukaryotes by 2DE, we applied two alternative approaches to identify PM proteins: 1) Apply PM proteins directly to SDS-PAGE, the lane was cut slices, digested, then the peptides mixture were separated by reversed-phase liquid chromatography MS/MS; 2) PM proteins were directly digested with CNBr, Lys_C and Trypsin followed by fractionation using strong cation exchange chromatography. Each of these fractions was analyzed by reversed-phase liquid chromatography MS/MS. These two approaches can overcome the drawback of 2DE, can improve the identification resolution of membrane proteins and allowed us to identify 537 unique proteins were identified, in addition to 82 protein groups, 131 proteins were clearly localized in plasma membrane and 15 were unknown. To our knowledge, this is the most comprehensive proteomic analysis of plasma membrane proteins in mammalian tissues to date. 131 PM proteins covered all of functional modular on PM, including lipid raft, signal transduction, membrane skeleton, cell contact, membrane fusion, transporter, lipid metabolism enzyme and so on. This result shows the methods we applied to identify PM proteins is unbiased and effective.

Additionally, through analysis of integral plasma membrane proteome, we find that the cytoskeletal proteins such as tubulin, fodrin, actin and myosin resist extraction with 0.1 M Na_2CO_3, suggesting these proteins were tightly associated with PM, and may play important roles in maintaining stability and signaling function of plasma membrane. And some cytoplasmic proteins such as HSP70, HSP27, HSP60, GRP78 and protein disulfide isomerase were identified in HFL PM in high abundance, these proteins has been verified to localize in PM by Hanash's lab. The results presented here provide global proteins expression profile in plasma membrane of HFL, and suggested SDS-PAGE, 2D-LC can effectively analyze PM proteome.

8.11

Characterization of Mitochondrial Proteome in Human Fetal Liver During 16–24 Weeks of Gestation

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The human fetal liver during 16–24 weeks of gestation is a major site of embryonic hematopoiesis and immune development in human being. Mitochondrial are ideal targets for global proteome analysis because they have a manageable level of complexity as a consequence of the their apparent procaryotic ancestry. what is the constitute of mitochondrial in the human fetal liver during 16–24 weeks? The research profile the human fetal liver mitochondrial proteome and try to optimize the separation and identification technology. In our study, using 2DE-MALDI-TOF-TOF and 2D-LC-MS/MS, we have established a reference database of 543 mitochondrial and mitochondrial-associated proteins of the human fetal liver in which 155 proteins were localized in mitochondrial definitely and 92 proteins not annotated. This profile currently provides the most comprehensive overview of the mitochondrial proteome in terms of the proteins' localization information as well as their functional classification. In summary, we provided a strategy for mitochondrial proteomics research. Identification of proteins from mitochondrial fractions using 2D-LC-MS/MS followed by bioinformatics annotation, which was proved as a high-throughput, sensitive and effective analytical approach for subcellular proteomics research.

8.12

Systematic Identification and Characterization of Human Fetal Liver Proteome—The Preview of Human Liver Proteome Project

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As the preview of Human Liver Proteome Project (HLPP), systematic identification and characterization of human fetal liver (HFL) proteome using a integrated strategy gave the birth of protein expression profile, phosphorylated protein profile (Phosphoproteome) and protein-protein interaction (Interactome) of HFL. Analysis of protein expression profile from HFL PM (plasma membrane), cytosol, mitochondria, nucleus and tissue lysate via CCPIT (comprehensive and complementary protein identification technology) resulted in the detection and identification of 64960 peptides and 24454 unique peptides corresponding to at least 2495 unique proteins. Combining immunoblotting with 2-DE and IMAC technology, we detected and identified at least 125 unique phosphorylated proteins (at least 109 proteins containing phosphoserine; 88 proteins containing phosphothreonine and 88 proteins containing phosphotyrosine) and 36 phosphorylation sites within the phosphoproteome of HFL. In order to describe protein interactions concerning HFL special function, Y2H, computational analysis, interaction database were used to build up the interaction network in HFL and interacting domain data were used to validate the computational results. A high confidence map of 729 unique interactions involving 684 proteins was obtained by these methods. Our initial analysis of the data describes some of the insights of HFL that can be glanced from these maps. This HFL proteome study would become the guarantee of the success of HLPP.

8.13

Study on Transcriptome of Human Fetal Liver Aged 22 Weeks of Gestation

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Liver is the largest gland in human body. We studied the 20282 ESTs coming from the cDNA library of human fetal liver to find new genes and genes families. At the same time, we construct an ORF database for protein identification in proteomic studies.

After the vector sequences were screened and repeated sequence were eliminated, the sequences were aligned with UNGIENE database to get frequency information. and aligned with NT database to classify the ESTs according to their scores and functions. Contig homologous sequences for unknown ESTs and confirm the results by comparing with original ESTs and four database: DOTS, unigene, MGC, and twinscan predicted transcripts. At the same time, contig integrity was validated and 5 ORFs of high reliability were acquired for each contig. Related protein database. was built The possible functions of all the extended ESTs were predicted by Prosite, PFAM, PSORT, SOSUI and electronic localization.

Finally we obtained 12374 non-redundant sequences, 2125 genes with known functions, 2800 genes with unknown functions, the extended sequences of the 2800 ESTs and their 13565 proteins for identification. 1500 full length ORF sequences were verified. 7411 protein with high reliability related to these ORFs were obtained and their functions were predicted. A protein identification system was built, and a database was constructed to collect all the information of the 20282 ESTs.

The prediction should be verified by experiments.

8.14

Plasma Proteomic Profiling and Identification of Circulating Biomarkers For Monitoring Fulminant Hepatic Failure in a Porcine Model

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Purpose: Fulminant hepatic failure (FHF) is associated with high mortality. Effective treatment is liver transplantation, which however, is costly and the patients require long-term immunosuppression. Therefore, there is a need to differentiate a group of patients who could be exempted from liver transplantation due to their potential of spontaneous recovery. Reliable biomarkers of FHF could facilitate in disease monitoring as well as to predict the irreversibility of FHF in combination with established clinical parameters. The purpose of this study is to study the plasma proteomic profiles by SELDI-TOF and to identify useful biomarkers for FHF by tandem MS/MS.

Material and Methods: Plasma was collected from pigs that received D-galactosamine (0.75 g /kg) before administration, day 1 and day 2 after drug administration ($N = 12$). Pigs were also treated by the selective plasma filtration, which is a liver support system, and the plasma was also collected from the recovered pigs. Plasma was then applied onto Ciphergen ProteinChip® H50 and IMAC3 arrays. Proteins bound to the chips were analyzed on a ProteinChip Reader Model PBSIIc. A SEDLI plasma protein profile was generated in which the individual proteins were displayed as unique peaks based on their mass and charge (m/z) and the resulting protein profiles were analyzed.

Results: Using the Ciphergen biomarker wizard® we were able to find a protein peak with the molecular mass of 8.3 KD that decreased remarkably and 13.8, 15.0 and 16.2 KD that increased significantly during the disease progression. In addition, they exhibited a linear relationship between the relative intensities of the peaks and the time of the disease progression. When the pigs recovered by the treatment of selective plasma filtration, the protein with 8.3 KD reappeared while the proteins of 13.8, 15.0 and 16.8 were degraded. By tandem mass spectrometry (MS/MS) fragmentation, the proteins were identified to be complement C5 (8.3 KD), serum amyloid A protein (13.8 KD), fatty acid binding protein (15 KD) and phospholipase A2 (16.2 KD), respectively. They were further confirmed by the SELDI protein chip immunoassay. The protein chip immunoassay also showed a decrease of complement C5 and increase of serum amyloid A protein, fatty acid binding protein and phospholipase A2 in the serum of a patient with liver failure compared with the normal control serum.

Conclusion: These findings suggested that these identified proteins could be used for monitoring FHF, especially for those due to drug overdose.

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8.15

Characterization of Nuclear Proteins in Human Fetal Liver During 16–24 Weeks of Gestation

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The human fetal liver (HFL) during 16–24 weeks of gestation is an organ in developmental stage, which has functions in metabolism of carbohydrates fats proteins and so on. Moreover, it is a major site of hematopoiesis in fetus. It will understand the mechanism in fetal liver by discovering the information of proteins in large scale through proteome research. In the proteome research, the low abundance protein is a bottle-neck. At present, there are two main solutions to deal with this problem: to deplete the high abundant proteins and protein fractionation. Based on the characteristics of eukaryotic cell structure, it is possible to fractionate proteins by organelles extraction. In our research, we established the technical platform of nuclei extraction through sucrose density gradient centrifugation. The purity was analyzed by western-blot, enzyme assay and electron microscopy, indicating that the nuclei were enriched more than 5 times. After the nuclei lysed, the nuclear proteins were further fractionated to hydrophobic, hydrophilic and pellet parts by kit. Further, the proteins were separated and identified by one dimensional electrophoresis and mass spectrograph. 422 proteins were identified in which 145 proteins were previously located in nucleus, 16 novel proteins (10 predicted to localized in nucleus) and 9 low abundant proteins in liver according to SWISS-PROT annotation. The identified nuclear proteins were structural and RNA splicing proteins, which were expressed ubiquitously among various cells. The tissue-specific classification of proteins demonstrated that many proteins were not only expressed in liver but also highly in testis and brain, suggesting that the proteins might play key biological role.

8.16

A Robust Platform for Comprehensive Liver Protein Profiling and Whole Proteome Arraying

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We have developed an integrated and high-throughput platform for proteomics research based on multi-dimensional liquid chromatography and tandem mass spectrometry to profile genome-wide protein expression associated with liver development and pathogenesis. The purpose of the multi-dimensional liquid chromatography is to address the dynamic range and sensitivity issues associated with protein analysis. Our first objective is to obtain high-resolution proteomes of mouse and human liver cells. At present, we have obtained 39,632 gene identifications (GI) raw data from mouse liver fractions and 14,578 raw GI from human liver fractions. After removing redundancy present in the database, there are 6,409 and 5562 unique GI respectively for mouse and human liver fractions.

The identified proteins have been automated clustering into GO (Gene Ontology) groups and the comparison between mouse and human liver proteomes can now be compared. These two proteomes are now constructed as database, integrated ITRI proteome database (i^2 PDB). Such a database is obviously a very useful resource for liver biology research.

As the information of identified proteins is fully annotated and integrated with our laboratory information management system so the protein components of various liquid chromatographic fractions are known. The various chromatographic fractions can be used as protein source for constructing whole proteome protein array. The potential applications of such an array will be discussed.

8.17

Dynamic Changes in Protein Synthesis and Degradation Related Proteins in Fibrotic Rat Liver and Effect of Fuzhenghuayu Recipe on It

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Being a wound healing process in chronic liver injury, hepatic fibrosis represents the common pathological basis for chronic hepatic diseases to develop into cirrhosis. Aiming at screening physiological and pathological condition related proteins and providing a new approach to early diagnosis and treatment for liver fibrosis, we investigate dynamical changes of proteome in normal and fibrotic rat liver and modulation effect of Chinese herbs on these changes.

With 4 rats in each group at 4 time points, 48 rats were randomly assigned to normal group, model group and Fuzhenghuayu recipe treatment group. The model rats were hypodermicly injected with 40%CCl_4-olive oil for 12 weeks at dose of 0.2 ml/100 g body weight and in frequency of twice a week. Rats in treatment group were orally given Fuzhenghuayu recipe from the first day of modeling. The rats in normal group, model group and treatment group were sacrificed in batches at the 4th, 8th, 12th and 16th week. Liver protein was extracted for two-dimensional electrophoresis and protein spectrum was analyzed with image analysis software (PDQUEST 2-DE). More than 30 differentially expressed protein proteins were identified, among them, proteins that relate to protein metabolism were L-PSP and ER-60 protease. As a strong inhibitor of protein synthesis, hepatic L-PSP expression in model group was significantly stronger than that of normal group and peaks at 4th and 12th week. Contrary to changes of serum total protein and albumin of fibrotic rats which reached its bottom and was significantly lower than that of normal rats ($p < 0.05$), L-PSP reached its 2 peaks at 4th and 12th week. The serum albumin level increased gradually while expression of L-PSP returned to normal level at 16th week with withdrawal of CCL_4 stimulation, indicating that hepatic cells injury results in decreased protein synthesis at 4th and 12th week. These results strongly suggest that L-PSP has some relationship to decreased protein synthesis during hepatic injury. L-PSP expression decreased significantly at each time points after Fuzhenghuayu recipe treatment, indicating that Fuzhenghuayu recipe can maintain normal protein synthesis by down-regulating L-PSP expression. Compared with modeling group, ER-60 protease, which has something to do with degradation of abnormal protein, showed a decreased expression in treatment group after 12 weeks modeling. We postulate that anti-fibrosis effect of Fuzhenghuayu recipe might relate to its effect on ER-60 protease and L-PSP expression, further study is expected to testify the preliminary conclusion.

8.18

Molecular Classification of Hepatocellular Carcinoma by Proteomic Expression Profiling

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Background: Hepatocellular carcinoma (HCC) is the second leading cause of cancer death in Hong Kong and the 5th worldwide. The prognosis for HCC patients is still poor because of the low curative resectability as well as high recurrence rate of the disease even after hepatectomy. Therefore, new technologies for earlier detection of pre-malignant stage of liver cancer are urgently needed for better prevention and treatment of HCC.

Methods: Proteomic expression profiles were generated by 2-D gel electrophoresis followed by silver stain and image capture analysis. A preliminary "training" set of proteomic profiles derived from 50 pairs of tumor and peritumor surgically fresh resected tissues with liver cancer were analyzed by the Classification and Regression Trees (CART) searching algorithm that identified a proteomic pattern that completely discriminated tumor from non-tumor tissues. The training set was then used to classify an independent set of 24 masked tumor/peritumor tissues from HCC patients plus 12 liver donor tissues from normal healthy subjects without any malignant disorders.

Results and Conclusion: The algorithm identified a proteomic classification pattern in the training set that correctly segregated the cancer from non-cancer tissues. The prediction accuracy for tumor and peritumor HCC tissues is 92.9%, whereas the pattern accurately discriminated all 12 normal liver tissues (100%) from cancer. Furthermore, a strong feature algorithm has also been developed and proven to be for the identification of novel biomarkers that can discriminate HCC cancer with high risk of recurrence. These findings justify a prospective assessment of proteomic expression patterns in surgical tissues and parallel comparison with plasma proteome datasets obtained from SELDI mass spectra for molecular classification of liver cancer.

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Dysregulation of Chaperone Proteins Hsp27, Hsp70 and GRP78 in Hepatocellular Carcinoma

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The purpose of this study is to establish differential protein expression profiles between hepatocellular carcinoma (HCC) and normal liver tissues by 2-D gel electrophoresis and MALDI-TOF. We aim to identify potentially useful tumor-associated markers that are dysregulated in HCC specimens. Fifty-two HCC surgical specimens and paired peritumor tissues were collected from patients underwent hepatectomy at Queen Mary Hospital, Pokfulam, Hong Kong, with approved protocol from the Institutional Ethics Committee. Cellular proteins were fractionated by differential extraction buffer system, and subjected to two-dimensional gel electrophoresis followed by MALDI-ToF analysis. Three chaperone heat shock proteins Hsp70, Hsp27 and GRP78 were found significantly dysregulated in 52 pairs of HCC tissues by proteomic analysis. These chaperon proteins are believed to play important roles in protein folding, transport and assembly as well as anti-apoptotic pathways in hepatocarcinogenesis. One-way ANOVA was used for statistical analysis, and $p < 0.05$ considered to be significant. Over expression of the chaperone proteins were confirmed by western blot. Clinical correlation of these chaperone proteins indicated their potential pathogenic roles in advanced tumor staging and metastasis, hinting for poor prognosis of patients with HCC.

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