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Report of the 9th HLPP Workshop October 2007, Seoul, Korea

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Abstract

The Human Liver Proteome Project is one of the Human Proteome Initiatives launched by Human Proteome Organization (HUPO). Major achievements of the project have been obtained under the efforts of international collaboration with all the participants since it was formally proposed in 2002. Its updated progresses were presented in the latest workshop held in conjunction with the sixth HUPO World Congress in October, 2007, Seoul, Korea. Furthermore, four topics related to the project as well as other initiatives were lively discussed among all the attendees.

Keywords

Human Liver Proteome Project; Human Proteome Organization; Workshop

1 Overview of the Human Liver Proteome Project (HLPP) and the latest HLPP workshop

The HLPP is a large-scale international collaborative initiative focusing on the proteomic analysis of the human liver. It aims to generate a comprehensive protein atlas of the human liver, uncover the proteomic basis of liver development, physiology and pathology and develop the liver-specific diagnostics and therapeutics.

Under the efforts of international collaboration with all the participants, we set up standard operating procedures (SOPs), optimized the strategies of the proteomic analysis and investigated the proteomes of the Chinese fetal liver tissues, French adult liver tissues, and Chinese adult liver tissues in turn.

Based on the past achievements, we held a workshop in conjunction with the sixth Human Proteome Organization (HUPO) World Congress on October 6 2007, Seoul, Korea. More than 100 participants from 14 countries attended the workshop and the progresses were

presented, respectively by Professors John Bergeron (McGill University, Canada), Fuchu He (Beijing Proteome Research Center, China), José Mato (CIC bioGUNE, Spain), Young-Ki Paik (Yonsei University, Korea), Chantal Housset (INSERM, France), Xiaoming Yang (Beijing Institute of Radiation Medicine, China), Victor Zgodá (Orekhovich Institute of Biomedical Chemistry, Russia), and Xuemin Zhang (National Center of Biomedical Analysis, China). In addition, Professor Bill Jordan (Victoria University of Wellington, New Zealand) introduced the Membrane Proteomics Initiative (MPI) and its latest progress. Subsequently, a lively discussion on four topics took place among all attendees.

As known well, liver is the headquarters of powerhouse and chemical manufactory, and the main provider of plasma proteins in human body. With the latest progress, especially the recent achievements in proteomics-based profiling of the whole liver tissues, the focus of HLPP has been shifted gradually from the normal liver samples to diseased ones, from the whole liver tissues to the hepatocellular organelles and different sorts of liver cells, and from the static expressions to dynamic changes. Proteomic analysis of the liver diseases, such as liver fibrosis, cirrhosis, and liver cancers as well as Nonalcoholic fatty liver disease (NAFLD) was one of the most important topics during this HLPP Workshop.

2 Progress of the HLPP subprojects

2.1 Sample collection and banking

In the HLPP pilot phase, the French team provided ten “normal” human liver samples, eight from patients with metastasis (>3 cm) and two from benign tumor in the light of the standard operation procedure. The sampling time of ischemia was less than 30 min, and the time before snap-freezing was $1 \text{ h} \pm 10 \text{ min}$. The Chinese team mixed and distributed the samples to eight laboratories. As reported by Professor Chantal Housset, the network for the national liver cancer bio-banks in France has been established and works well all over the France. Frozen samples were stored in each center and related annotations were collected within a central database, under the high assurance-quality procedures according to international standards and ethical rules. By May 2007, 902 liver samples from 740 patients with standardized clinical, laboratory, and therapeutic information and informed consents had been collected.

2.2 Protein expression profiling

Professor Fuchu He reported the latest progress of the protein expression profiling. By using an integrated separating-identifying platform, 6788 proteins were identified in 95% confidence with at least two peptides matching in the Chinese human liver samples, including 3721 proteins newly found in liver with 977 hypothetical proteins. Meanwhile, the Chinese Human Liver Transcriptome (HLT) dataset was established, which comprised of 11 205 protein-coding genes. After primary analysis, the large data were re-analyzed more deeply, leading to interesting and exciting conclusions. For example, there appeared to be a qualitative and quantitative coordination between Human Liver Proteome (HLP) and HLT. The patterns of chromosomal distribution of the genes corresponding to HLP were highly consistent with those of HLT. Both the detection rate and abundance of proteins in biosynthesis increase gradually along with the genetic information flux, from replication to

transcription and translation, further supported by the data analysis on the cell cycle regulators. Based on the comprehensive annotations of GO slim, protein domain, signaling transduction, transcription factor, and the comparison with plasma proteome, the dataset has provided the proteomic insight of liver physiology.

2.3 Protein–protein interaction mapping

Systematical mapping of human protein–protein interactions have been conducted, but it is far from completed. One major goal of HLPP is to study binary protein–protein interactions between liver proteins and the important liver protein complexes. Reported by Professor Xiaoming Yang, arrays of about 5000 unique ORFs were screened by yeast two hybrid mating method, and more than 1000 potential interactions were identified to date. More than 55% of 54 random selected interactions were further verified by co-immunoprecipitation assay. Interestingly, a few protein complexes were identified by affinity method, which focused on HBV-related proteins and enrichment of liver-specific transcription factors. More than 100 proteins associated with HNF, AKT, and HBx, were identified by MS with high confidence.

2.4 Protein localization mapping

Professor Victor Zgoda introduced two ways to analyze subcellular protein localization, silico predictive approach with the accuracy up to 70%, and centrifugation combined with immunochemistry. These data indicated high confidence of experimental and predictive results for 80–85% of cytosolic and microsomal proteins. Other 15–20% proteins with dual locations could be explained by the contaminations of fractions or nature of these proteins. In order to examine the localization of proteins in mammalian cells on large-scale or global analyses, Professor Xuemin Zhang developed a new system for comprehensive protein localization analysis, a cell-based high-throughput localization array. More than 1000 proteins could be localized and screened at one time in sub-cellular level using this system. This system had been used to examine the colocalization of multi-proteins, discover the transforming factor closely-related to hepatic cell tumorigenesis, and screen the interaction between different proteins in mammalian cells, such as PIAS3 and PRB. Worth to mention, in screening substrates of ubiquitin- proteasomes pathway with this system, 112 proteins were tested and 24 candidate substrates, such as MAPKAPK3, NLK, and RhoGDI2, identified as novel target of ubiquitin.

2.5 PTM profiling

The reversible phosphorylation of proteins is recognized as one of the important PTMs in regulation of cell signaling and function of biological systems. To tackle the technique challenge and find potential diagnostic or therapeutic phosphorylated biomarkers for hepatocellular carcinoma (HCC), a comprehensive 3-D nano-LC system (SCX/TiO₂/C18) system as well as the PF2D-based 2-D protein or peptide separation platform were established in Professor Young-Ki Paik's laboratory. The 3-D system appeared to be effective especially when combined with linear IT (LTQ) and neutral loss dependent scan for phosphopeptide detection. Exciting data were obtained when this system was further applied to profile and compare the phosphoproteome of HCC with that of noncancer tissues,

the culture media or cell lysates of HCC with that of noncancer liver cell lines, and plasma from the HCC patients with plasma of normals.

2.6 Proteomic analysis of liver diseases

In addition to the sample collection and distribution, Professor Chantal Housset reported the comparison of the proteome of the tumor and nontumor areas of liver samples, which were isolated by using laser microdissection (LM). Differential protein profiling exhibited noticeable differences between tumor and nontumor: 30% of the protein spots with deregulated expression in tumorous LM-samples did not display any modification in homogenates; conversely 15% of proteins altered in tumorous homogenates were not impaired in LM-hepatocytes. Professor Housset also generated an animal model of DEN-induced liver cirrhosis and HCC. The process of NAFLD includes steatosis, steatohepatitis, and liver fibrosis. About eight million people in the European Union may have nonalcoholic steatohepatitis (NASH), 25% of which may progress into liver cirrhosis, and 10% cirrhosis might lead to HCC. Professor José Mato's laboratory integrated metabolomic profiling to identify the NAFLD biomarkers, and compared the protein profiling of steatosis with that of control, or protein profiling of steatosis type 1, type 2, and control. Differentially expressed proteins between steatosis/NASH and control were identified by DIGE analysis of liver biopsies. Nearly ten proteins were validated by Western blotting. NAFLD animal models were also generated for biomarker discovery, including MAT1A deletion (inducing steatosis, NASH, and HCC), GNMT deletion (resulting in steatosis, fibrosis, and HCC) and CD81 transgenic mice (spontaneously developing steatohepatitis).

2.7 Animal models

The ER plays an important role in the secretory pathway of the cell, and also it is an important site for lipid and steroid oxidation and drug detoxification. The report from Professor John Bergeron displayed their efforts on the comprehensive profiling of the mouse liver ER and Golgi. By using a label-free quantitative proteomics strategy, a parallel strategy just similar to that of SAGE profiling, his team has successfully performed relative quantification on all of the protein components found in ER and Golgi. Cluster analysis based on the quantification information revealed that the expression of some protein cluster was consistent among rER, sER, and Golgi. Taking the cytochrome P450 family as an example, Professor Bergeron showed the practicability of the proteolytic peptide in distinguishing highly homologous protein cluster and in correlating expression level between protein and transcript. In addition, as mentioned above, animal model of DEN-induced liver cirrhosis and HCC, and NAFLD animal models were well established.

3 Discussions and future plans

Following discussions focused on four major topics were led by Professors Young-Ki Paik, Xiaohong Qian, John Bergeron, Bill Jordan, Rolf Apweiler, José Mato, and Pengyuan Yang, respectively.

3.1 Technology strategies

The conclusion that, as a general consensus, both optimizing present technology platform and developing new technologies should be as significant objects for HLPP was made from the discussion on “Technology strategies” led by Professors Young-Ki Paik and Xiaohong Qian. Different results may be obtained by using different Mass spec, such as LTQ-FT or Q-TOF, pointed by Professor Qian. The major challenge to identify PTM proteins is that the modifications are seldom stable. Actually, most of the modifications are always in change. The dynamic changes increase the difficulties in the analysis of the PTM proteins. Another challenge is that the phosphorylated proteins are low abundant, said Professor Xiaohong Qian. Her group has investigated the phosphorylated proteins in liver cell line to evaluate the technique routes. Nearly 200 phosphorylated proteins have been found. In order to identify more phosphorylated proteins, enriching the low proteins, and repeating the experiments several times as well as analyzing the same samples with different mass spec would be most important.

Professor Helmut Meyer emphasized that it was very important, for obtaining the right samples, to keep a good collaboration with the surgeons who could give a right diagnose, and get right human samples. In the summary of the topic, Professor Paik said all of us had realized that it was necessary to develop technologies to investigate the proteome, especially for the PTM and other evaluation as well as the validation. The focus would be on what we could target or how we could do it and how to take advantage of the different technologies to find the common or different modifications in liver proteome. He suggested that the target for liver disease or therapy should be set up, and we need to go over the PTM technologies with different choice of value.

3.2 HLPP datasets

The second topic discussion was chaired by Professor Rolf Apweiler. There were three proteomic datasets generated from the HLPP so far. The human fetal liver and the French human liver data were submitted to the EBI and open to the public, and the Chinese normal liver data were being quality-controlled, integrated, and analyzed, then would be submitted to the EBI as soon as possible. Besides the databank of the protein expression profile, Professor Fuchu He said, those for protein–protein interaction, localization and the PTM profile were being built, and some data have been generated and integrated. At the end of discussion, Professor Apweiler suggested that all the data produced from the French human liver samples should be integrated and open to all as soon as possible. It was essential for the HLPP to confirm what is the most important and what station is based as soon as possible.

3.3 Protein validation

In the discussion session on protein validation, which was chaired by Professors John Bergeron and Bill Jordan, Professor Bergeron briefly introduced a globally distributed project, of which results showed trivial interaction with each laboratory. He mentioned every initiative has generated hundreds of favorite proteins, and emphasized the importance of the antibodies in the validation of the proteins.

3.4 Strategy for the proteomic analysis of liver diseases

The last and hot topic was “the strategy of the proteomic analysis of liver diseases” led by Professors José Mato and Pengyuan Yang. 2-DE and MS were mostly used for the discovery of potential biomarkers related to the liver diseases, especially for the HCC, Professor Pengyuan Yang said. Not only the liver tissues, but also the body fluids, especially the blood or serum from the patients suffering from the liver diseases should be investigated as the key points. In the next stage of HLPP, more than 100 potential biomarkers might be discovered and provided for clinical application. Furthermore, the comparison of the proteomic data of liver tissues with the blood samples should be carefully considered. As there were many same proteins in the samples from different patients, Professor Helmut Meyer appealed to establish the SOPs for the proteomic analysis of liver diseases, from the sample collection to preparation, storing, and distribution, as well as the SOPs for the protein validation.

4 MPI

The MPI was initiated by Asia-Oceania HUPO (AOHUPO) and chaired by Professor Bill Jordan. In his presentation, Professor Jordan introduced the goals of the project, which were to develop methods for characterization of membrane proteomes and the proteomes of specific membrane systems. The initial strategy was to catalog TM proteins, test, and optimize the performance of proteomic analysis techniques of the MPI standard. In the first phase, 17 laboratories were involved in the mouse model studies, and the integral membrane proteins of liver microsomes from male C57BL/6J mice (10–11 wk) have been enriched. So far, the first set of the data was generated by the MPI and would be collected and integrated for bioinformatic analysis soon.

Abbreviations

HCC	hepatocellular carcinoma
HLPP	Human Liver Proteome Project
LM	laser microdissection
MPI	Membrane Proteomics Initiative
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
SOP	standard operating procedure