

HUPO HUMAN LIVER PROTEOME PROJECT (HLPP)

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Planning Committee

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I. Introduction

At the Bethesda workshop of the Human Proteome Organization (HUPO), 28-29 April, 2002, there was broad interest and strong voice for a Human Liver Proteome Project (HLPP) under HUPO auspices, with international academic, industry and government participation. To follow up this initial proposal at the workshop, HUPO hosted the HLPP Workshop in Beijing from Oct 22 to 24, 2002 in which more than 100 experts and the representatives from China (mainland, Hong Kong and Taiwan), South Korea, Canada, France, the United States, Germany, Russia, Japan, Thailand, United Kingdom, Australia, and New Zealand actively participated. From this workshop, there has been general consensus on the scientific goals and future direction of HLPP. At HUPO First World Congress in Versailles from November 21 to 24, Fuchu He, as an organizer of HUPO HLPP Workshop in Beijing, gave a brief report to the participants in the HUPO congress as well as HUPO council members. During the HUPO Congress, there was a special group meeting for HLPP on November 23, in which Drs. Fuchu He (China), John Bergeron (Canada) and Christian Brechot (France) were nominated as the Co-Directors of HLPP. We now announce the statement on the initiation of HLPP as follows.

Your participation to this project and comments are welcome. If you wish to join this project, please fill out the survey form and return it to Dr. Fuchu He (hefc@nic.bmi.ac.cn) by February 28, 2003. The HLPP Committee will announce the potential participating laboratories to HLPP on the web site (www.hupo.org) after peer reviewing their survey forms on the basis of certain criteria.

II. Scientific Background

The liver is the largest organ in the body, second only to the brain in organ complexity. It displays the main digestive function for the metabolism of most substances; and produces various plasma proteins. It is the most effective site for detoxification of xenobiotics, phagocytosis of solid material, and for the guardian interposed between the digestive tract and the rest of the body. Liver

is also the ultimate source of a major portion of the fatty acids to other organs used as the primary source of energy in the fasted state. It plays a major role in determining the pharmacokinetics of a drug, because it is the major organ of drug elimination by the metabolic capacity and biliary excretion, and also the major site to influence the distribution because of the synthesis of binding proteins of drugs.

Liver diseases, such as viral hepatitis, liver cancer, alcoholic and drug-related liver injury, are great challenges for modern medicine. Worldwide, there are over 350 million hepatitis virus-carriers, and over a million deaths per year (about 10% of all deaths in the adult age range) can be attributed to viral hepatitis and even more deaths resulted from liver fibrosis, steatosis, and cirrhosis associated with viral hepatitis. Worldwide, over a million deaths per year (also about 10% of all deaths in the adult age range) can be attributed to hepatocellular carcinoma (HCC). What is more, the number of world-wide deaths due to viral hepatitis and/or HCC is increasing dramatically. Thus, the global comprehension of the molecular basis of liver and its diseases is pressing for human beings. For this purpose, proteomic analysis is one of the best alternative strategies.

Transcriptome is essential basis for proteomic analysis of a given tissue or cell line. Because of its biological, physiological and pathological significance, the serious transcriptomes of human liver, such as fetal liver, adult liver and liver cancer were well established. Those transcriptomes of human liver could provide valuable resources not only for integration (data-based) with corresponding proteome but also for scaling protein identification (data-based), subcellular localization and protein linkage mapping (cDNA-based).

III. SCIENTIFIC OBJECTIVES of the HUPO HLPP

- 1. Generation of Compiled Expression Profile of Liver (Proteome):** Comprehensive analysis of human liver protein constituents in health and disease states.
- 2. Establishment of Subcellular Localization Profile:** Determination of protein localization and Profile of Sub-cellular proteome.
- 3. Networking of Liver Proteins Linkage Map (Interactome):** Comprehensive analysis of liver protein-protein interactions and networks of liver proteome.
- 4. Elucidation of Protein Modification Profile:** Systematic analysis of post-translational modifications of liver proteome
- 5. Bridging Liver Proteome Project and Plasma Proteome Project.** Parallel coordination of these two initiatives with respect to resources, technology, and knowledge database will be achieved in order to discover biomarkers.
- 6. Construction of a Knowledge Database:** Integration and correlation of human liver proteome with liver transcriptome and human genome.

IV. Resource development goals

1. Identification of sources of liver samples for analysis to be shared among investigator groups

- for comparative analysis of standard specimens
2. Provision of one or more reference specimens.
 3. Development and assessment of tools for liver protein analysis, including creation of antibody panel for liver proteins and further antibody protein arrays.
 4. Development of a format for database(s) of liver protein expression, liver protein interaction map, protein modification profile, subcellular proteome, bridging of liver transcriptome and its proteome with human genome, and integration of liver transcriptome with liver proteome and plasma proteome.
 5. Establishment of groups committed to a collaborative HUPO-organized liver proteome project.

V. Initial Timeline

1. Oct. 22-24 2002: Hold a Workshop in advance of the Versailles HUPO Conference (November, 2002) to assess current capabilities, limitations, strategies, and preferences; to determine high-priority scientific and technical advances needed; to stimulate and begin to plan significant collaborations; and to assure adequate funding in cash and in-kind contributions.
2. November 21-24, 2002: A discussion panel on the Pre-Planning Committee Meeting on the Liver Proteome Project (HLPP) was discussed in the Versailles HUPO Congress. Drs. Fuchu He, John Bergeron and Christian Brechot were assigned as the world-wide coordinators (co-chairs) of Human Liver Proteome Project and authorized to set up the Planning Committee in tow months. Dr. Fuchu He was chose as the director and correspondent of HLPP.
3. During 2003: Achieve initial goals established at the Workshop and Versailles Conference.

VI. Priorities for the Action Plan in the First Year (2003)

1. Evaluate available specimen (tissue or parenchymal cell-hepatocyte, histological location-hepatic lobes, developmental stage, age/aging, gender and menstrual cycle, fasted/fed, common medications, selected diseases, etc.). Apply various cellular or subcellular fractionation, protein separation, protein subclass identification, 2DE, 2DLC, mass spectrometry, protein chip, and other analytic approaches. Compare and discriminate dynamic range or variation of proteins detectable. Link with HUPO work groups on Plasma, Antibodies, New technology and Bioinformatics.
2. Start with a protocol for technical features: Assess alternative methods of surgery or sampling, sensitivity of detection of low-abundance proteins, predictability and reproducibility of findings, effects of freeze/thaw cycles Specify gel-based and liquid-phase separation criteria. Define parameters for high throughput link to mass spectrometry for handling large numbers of samples.
3. Evaluate alternatives for depletion of high abundance liver proteins; invite companies or labs to provide antibodies, columns, protocol, and evidence; organize head-on-head comparisons in independent labs.s.
4. Estimate sensitivity of various analytical methods and set credible goals for detection of

low-abundance proteins in liver. Refine initial estimates of 1-10 fmoles for mass spec and 100 pg/ml liver proteins for gels, aiming to detect proteins in concentrations of 10^{-10} M. Compare with sensitivity of immunological detection (antibody arrays and ELISA assays).

5. Limit biological variability in the initial studies of assessing techniques. Identify reference specimens from Asian, Caucasian, and African populations.
6. Decide what weight of standard specimen should be distributed—initial estimates: 1.0 gram liver per lab x 30-50 expected cooperating HUPO labs, so need 50-80 gram whole liver.
7. Propose criteria for “counting” proteins—gene product, identifiable isoforms from post-translational modifications or assembly, spots or fractions from separation methods. Link to database development. Clarify meaning of “identifying 3000 (or 5000) proteins” in liver.
8. Determine abundance histogram with liver by mass range: peptides ≤ 1.0 kD, peptides/proteins 1.0-10.0kD (fractionate with MeOH, TCA, or TFA), proteins ≤ 60.0 kD (kidney filtrate); proteins >60.0 kD.
9. Provide guidance for meeting IRB requirements, HIPAA requirements, and similar regulatory regimes in various countries, as well as a framework and potential template contracts for managing intellectual property and conflict-of-interest issues. Specify information desirable for characterization of origin of specimens in specimen banks, both existing sources and new sources (date of draw, birth date, fed/fasted, smoking, alcohol, infections, drugs, menstrual stage, diagnosis, handling of the specimen).

VII. Broad Areas for the Phase II Plan (beginning Year 2)

1. Refine parameters of assays of various kinds: sensitivity, specificity, true/false positives, cost, automatability, through-put.
2. Integrate liver proteome with liver transcriptome.
3. Compare liver proteome with plasma proteome (coordinate with Plasma Proteome Project).
4. Annotate Human Genome with liver proteome.
5. Initiate Liver Proteins Linkage Mapping.
6. Establish modification Profile of liver proteins, such as phosphorylome and glycosylated proteome of liver
7. Set up subcellular localization profile of liver proteome
8. Expand disease-related studies, utilizing advances from phase I and related work.
9. Utilize animal models to restrict genetic, dietary, pharmacological, and other variation in studies of liver physiology and pathology.

Survey Form:

COMMENTS, SUGGESTIONS AND INTEREST IN PARTICIPATING INHUPO HUMAN LIVER PROTEOME PROJECT

Name of Principal Investigator

Organization:

Title:

Email:

Phone:

Fax:

Address:

Brief Description of Expertise/Current Research Interests:

Proposed Roles in First Year Plan:

Address specific priorities 1-9:

Share methods and results:

Provide reagents:

Share data:

Become a HUPO designated HLPP lab:

Become a corporate sponsor:

Existing and Future Grant Sources Supporting Your Works in HLPP

Other comments and suggestions welcomed.

Date:

Deadline for Receiving the Survey Form: Feb 28, 2003

Note: Please return to Dr. Fuchu He (Fax: 8610-68171208; email: hefc@nic.bmi.ac.cn)