

ICBC NEWSLETTER

INTERNATIONAL CANCER BIOMARKER CONSORTIUM

Dear ICBC Team Members:

It has been a busy fall for ICBC collaborators with two regional workshops conducted in September, a glyco-capture workshop hosted by Dr. Myeong-Hee Yu at KIST and a liver cancer workshop hosted by Dr. Hyang-Sook Yoo at KRIBB. Reports from those meetings are available in this month's newsletter, as well as a technology update, from Dr. Steve Carr and members of his lab on PEPPer (Platform for Experimental Proteomics Pattern Recognition), a suite of algorithms designed to make high quality liquid chromatography/mass spectrometry (LCMS) data more addressable, akin to microarray data.

Also this month, a new version of CPAS has just been released. Version 1.6 contains many key enhancements, such as features for project management and collaborations and improvement to tools for MS/MS analysis. The team highlight for this issue is the Hong Kong liver cancer team, led by Drs. John Luk and Sheung Tat Fan.

I would also like to bring to your attention that the next US HUPO meeting will be held in Seattle on March 4-8, 2007 and may provide an opportunity for ICBC members to meet or hold workshops.

Finally, I would also like to remind everyone to register for the upcoming meeting in Singapore Dec. 1-3, 2006 by November 1, 2006. A link to the registration is provided in this issue.

Best regards,
Lee Hartwell
President and Director
Fred Hutchinson Cancer Research Center

SCIENTIFIC UPDATES

PEPPer: Platform for Experimental Proteomics Pattern Recognition

Dr. Jacob Jaffe, Dr. D.R. Mani and Dr. Steven Carr

The introduction of DNA microarray technology to molecular biology has been revolutionary for genome-wide studies of expression, mutation, and chromatin. As such, it has made major contributions to our

fundamental understanding of human disease, and cancer in particular (1). Microarray experiments have discovered markers able to classify specific cancer types and identified causative mutations. Two important properties of microarrays that have allowed their adoption is that they are ab initio designable and addressable. A researcher may design a specific microarray to suit a particular scientific question, and genes of interest can be readily 'looked-up' via their physical positions on the microarray. A considerable backplane of analysis tools has also been developed to aid in analysis of large-scale data associated with microarrays.

Unfortunately comparable technology does not yet exist for similar interrogation at the protein level. Most proteomic biomarker discovery efforts are of the 'shotgun' design, where there is considerable difficulty in identifying and quantifying potential targets consistently across large sample sets. The Platform for Experimental Proteomics Pattern Recognition (PEPPer) seeks to make this process more robust (2). PEPPer is a suite of algorithms designed to make high quality liquid chromatography/mass spectrometry (LCMS) data more addressable, akin to microarray data. These algorithms are suitable for use with both 'label-free' approaches and strategies that involve isotopic labeling of proteins and peptides. The algorithms take as input the high resolution 'patterns' derived from mass spectrometry data collected on high performance mass spectrometers. This process allows the leveraging of the considerable array of marker discovery and analysis tools developed for the microarray world.

The first algorithm in the suite, Landmark Matching, allows time-base independent propagation of peptide identities across multiple LCMS experiments. Many researchers are familiar with the undersampling problem for peptide identification in proteomics experiments. Landmark Matching seeks to offset this by using the highly accurate mass assignments made from suitable mass spectrometers and relative retention time ordering to efficiently map peptide identities among a set of related experiments. This approach is similar to the AMT one proposed by Smith and colleagues at PNNL (3), but differs in that time is treated in a relative manner that allows for different experimental designs for deep coverage and quantification experiments. Landmark Matching on its own provides an enhanced capability for biomarker discovery in proteomic data sets.



October 2006
(Vol. 2, No. 5)

IN THIS ISSUE: Scientific Updates

Recent Publications

Collaborative Software

Administrative Updates

Biomarker Team Spotlight

Key Web Pages at FHCRC



The second algorithm, Peak Matching, allows the coalescence of unidentified mass spectrometry features that are likely to be the same across multiple experiments into recognizable groups, assigning them a computational identity. Peak Matching employs advanced clustering algorithms to achieve this task and greatly expands the number of features that are able to be quantified in large data sets. This further enhances the ability to perform marker discovery with existing tools suitable for microarray analysis. Importantly, the retention of high accuracy mass information with the features allows rapid identification of potential novel markers via targeted proteomics. The PEPPER suite will be available shortly as a module of GenePattern. Found at

<http://www.broad.mit.edu/cancer/software/genepattern/>.

References:

1. Golub, T.R., Slonim, D.K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J.P., Coller, H., Loh, M.L., Downing, J.R., Caligiuri, M.A., Bloomfield, C.D., and Lander, E.S. (1999) Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 286, 531-7
2. Jaffe, J.D., Mani, D.R., Leptos, K.C., Church, G.M., Gillette, M.A., and Carr, S.A. (2006) PEPPER: A platform for experimental proteomic pattern recognition. *Mol Cell Proteomics*
3. Smith, R.D., Anderson, G.A., Lipton, M.S., Pasatolic, L., Shen, Y., Conrads, T.P., Veenstra, T.D., and Udseth, H.R. (2002) An accurate mass tag strategy for quantitative and high-throughput proteome measurements. *Proteomics* 2, 513-23

CPAS 1.6 is now available!

CPAS Release 1.6 has just been launched with many key enhancements. In addition to new features for project management and collaborations, improvements have been made to the tools for MS/MS analysis including:

- Quantitation tools
- Ability to concurrently search runs as a group and individually
- Retention time display for peptide elution information

- A legend for fixed and variable modifications used in search parameters
- New format options for exporting processed data
- Usability improvements

In addition, CPAS now acts as a caBIG server in order to meet the needs of the National Cancer Institute. To learn more about CPAS Release 1.6 or to download a copy, please visit

<https://cpas.fhrc.org/Project/home/home.view>.

RECENT PUBLICATIONS

On occasion we will feature select recent publications that we think are of interest to ICBC members. The following paper reports a complex proteome in urine with the enrichment of membrane proteins. This article was recommended by Mandy Paulovich. If you notice an article you think would be of broad interest to ICBC members please send it to us.

[The human urinary proteome contains more than 1500 proteins, including a large proportion of membrane proteins.](#)

October MCP: A special issue on Clinical Proteomics

The October 2006 issue of the journal *Molecular and Cellular Proteomics (MCP)* will focus specifically on biomarker discovery and clinical proteomics. This is the fourth special clinical issue produced by the Journal, and it is available free to the public on the MCP website (www.mcponline.org).

The issue, which was compiled by guest editors Steven A. Carr and Julio E. Celis, contains mostly invited contributions, derived in part from presentations at the 2005 Asilomar Conference on "Biomarker Discovery and Validation: from Bench to Bedside" organized by Steve Carr and Leigh Anderson. Four research reports selected from direct submissions to the Journal are also included.

Recent Publications from Consortium members

N. Chignard, S. Shang, H. Wang, J. Marrero, C. Bréchet, S. Hanash and L. Beretta (2006) Cleavage of endoplasmic reticulum proteins in hepatocellular carcinoma: detection of generated fragments in patient sera. *Gastroenterology* 130: 2010-22.

J.T. Feng, S. Shang and L. Beretta (2006) Proteomics for the early detection and treatment of hepatocellular carcinoma. *Oncogene* 25:3810-17.

COLLABORATIVE SOFTWARE

None at this time.

ADMINISTRATIVE UPDATES

Report from the Liver Cancer Workshop – September 18-19, 2006 in Daejeon, Korea

Approximately 30 individuals from Hong Kong, Taiwan, FHCRC and Korea participated in the Liver Cancer Workshop hosted by Dr. Yoo in Daejeon, Korea. Intensive discussions centered on the method for glycopeptide capture and the preparation of the glycoprotein, MS equipment, analysis methods, and comparison of transcription profiles versus protein expression profiles. Participants shared methods for identification of HCC biomarkers and biological validations, and discussed other things, such as the sharing and comparison of results from cDNA expression profiles and protein expression levels, the sharing of results from validation of HCC biomarkers and implementation of co-publication through a collaboration system, and developing a method for collaboration in the identification of biomarker candidates using HCC model tissues and mice via a visiting researcher exchange program. The group established that videoconferencing would be the method used for communication among the collaborative teams, and that periodic progress reports on each team would be distributed in the form of a newsletter. The workshop participants are planning to meet, if possible, every 6 months or annually. Possible next meetings are April 2007 in Hong Kong or October 2007 before the 2007 HUPO meeting in Seoul, Korea.

Report from the Glyco-capture Workshop at the Functional Proteomics Center, KIST, Seoul, Korea, September 18-22, 2006

Over a dozen ICBC members, representing teams from Korea, Hong Kong, China and the United States attended the Glyco-peptide workshop hosted by Dr. Myeong-Hee Yu in Seoul, Korea. The workshop included the following lectures: Overview of Glyco-capture (Wei Yan); Trans Proteomic Pipeline (Mi-Youn Brusniak); Proteomics for Translational Modification Analysis (Cheolju Lee); Basic Principles of FT-ICR Mass Spectrometer (Sang-Won Lee) and Label Free Protein Quantification Method (Mi-Youn Brusniak).

Registration deadline for the December ICBC Meeting in Singapore is November 1!

This is a reminder to register for the December ICBC meeting in Singapore. The registration deadline is **November 1, 2006**. Each existing ICBC team is encouraged to bring up to 6 team members. Developing teams are encouraged to bring up to two representatives. There is no registration fee for this meeting; however, each individual is responsible for the cost of their airfare, ground transportation, and hotel. You may register on-line at the following link: http://www.fhcrc.org/science/international_biomarker/meetings/2006/dec/

* Please note that registering for the meeting does not automatically register you for your accommodations. You must go to the [Location/Accommodation](#) link to register for the hotel.

We look forward to seeing you in Singapore. Once the agenda is finalized, it will be posted on this website and a notice will be sent to meeting attendees. If you have questions, please contact me at kkreizen@fhcrc.org or Gail Campagna at gcampagn@fhcrc.org.

BIOMARKER TEAM SPOTLIGHT

Project Title: Investigating the Biomarkers for Diagnosis and Prognosis of Hepatocellular Carcinoma

Cancer Site(s):	Liver
Principal Investigator (s):	John M. LUK, Dr.Med.Sc. (Co-PI) Sheung Tat FAN, M.D., Ph.D. (Co-PI)
Participating Institutions:	University of Hong Kong (HKU – Coordinating Center) Centre of Cancer Research Centre for the Study of Liver Disease Genome Research Centre Department of Clinical Oncology Department of Chemistry Department of Medicine Department of Surgery The Chinese University of Hong Kong (CUHK) Queen Mary Hospital (QMH)
Clinical Samples:	Tissue banks
Animal Model(s):	For in vivo validation of HCC biomarkers (Yang, Z.F. et al., Cancer Research 65(1):219, 2005) For in vivo lead target evaluation (Liu, Y. et al., Cancer Research 65(9):3691, 2005)
Technical Approaches:	2-DE PAGE/ DIGE tissue profiling and tandem MS/MS Deep™ serum profiling and SELDI-ToF MS Cellular membrane proteins and glycopeptide enrichment Tissue microarray Hybridoma and phage-display antibody library

Brief Description of Project:

Objectives & Deliverables:

- To identify potential biomarkers for HCC, liver inflammation, and cirrhosis.
- To test and evaluate prognostic values of candidate biomarkers for HCC malignancy.

- To validate the markers for HCC in animal models and in vitro assays.
- To identify and characterize key cellular and signaling molecules in HCC.
- To develop monoclonal antibodies and lead compounds of diagnostic and/or therapeutic potentials targeting HCC.

Hepatitis B virus (HBV) infection is one of the most common viral infections in humans. The sequelae of chronic HBV infection, such as hepatitis, cirrhosis and hepatocellular carcinoma (HCC), are determined by the dynamic interaction between the virus and the mainly genetically-determined host response. Approximately 2 billion people have been infected with HBV and 350 million of them became chronically infected, mostly in the Asia Pacific region. Among them, around 25-40% will eventually die of liver diseases (viz. cirrhosis with or without hepatocellular carcinoma). Patients with HCC are often presented at late stage of the disease at the time of diagnosis. Examination of the alpha-fetoprotein (AFP) level has been proven to be an effective way to diagnose HCC and its recurrence. However, about 30-40% of HCC patients in China are AFP negative. Besides, there are a large number of patients with hepatitis and cirrhosis who are AFP false positive, rendering this marker less accurate and ineffective for HCC. As such, there is an urgent need to pursue a unique malignancy biomarker in diagnosing AFP-negative HCC and excluding those AFP false positive cirrhotic patients.

The objective of this study is to identify biomarkers for diagnosis and prognosis of HCC as well as for monitoring disease progression by employing proteomic approach. Today, we have analyzed over 100 pairs of HCC tissues and adjacent non-tumor tissues by 2-DE, with 20 healthy liver samples (residual graft) as normal control subjects. Furthermore, serum profiling of the corresponding serum samples were assayed by SELDI-ToF-MS system. Clinical correlation of the proteome profiles or selected biomarkers with defined pathological features (e.g. tumor staging, hepatitis, microvessel density, venous infiltration, recurrence and overall survival) are under intense investigation.

Protein spots identified on a 2-D gel matrix of cancer proteome will be picked up as potential HCC biomarkers or targets for biochemical identification and characterization using MS/MS (Q-STAR and ABI4700). Phage or hybridoma monoclonal antibody (mAb) against these proteins will be generated for screening the specificity as HCC biomarkers by Western blot and immunohistochemistry. Our team has successful record in designing mAb or its derivatives for diagnostic assays and other clinical applications (with a number of US patents filed). We will examine the usefulness of these specific mAbs conjugated with chemotherapeutic agents and potential lead compounds to eradicate HCC tumors in animal models, prior to further prospective clinical trials.

Team Members and Expertise:

Chi Ming CHE, Ph.D. (Chair Professor of Chemistry, HKU-Key Investigator) [cmche@hkucc.hku.hk]
 Sheung Tat FAN, M.S., M.D., Ph.D. (Chair Professor of Surgery, HKU-Biomarker Team Co-PI) [hmsfst@hkucc.hku.hk]
 Dr. X.Y. GUAN (Clinical Oncology, HKU)
 Dr. Q.Y. HE (Institute of Molecular Biology, HKU)
 Professor P.B.S. LAI (Surgery, CUHK)
 George K.K. LAU, M.D. (Associate Professor of Medicine, HKU-Key Investigator) [gklau@netvigator.com]
 Dr. P.Y. LEE (Surgery/Medicine, HKU)
 John M. LUK, Dr.Med.Sc. (Associate Professor of Surgery, HKU-Biomarker Team Co-PI) [jmluk@hkucc.hku.hk]
 Dr. W.W. MAK (Genome Center, HKU)
 Irene O.L. NG, M.D. (Professor of Pathology, HKU-Key Investigator) [iolng@hkucc.hku.hk]
 Dr. R.T.P. POON (Surgery, HKU)
 Dr. T.C.W. POON (Medicine, CUHK)
 Dr. A. SIU (Chemistry, HKU)
 Professor P.K.H. TAM (Surgery/Genome Center, HKU)
 Lap-Chee TSUI, Ph.D. (Vice-Chancellor, HKU-Key Investigator) [tsuilc@hkucc.hku.hk].

Core Facilities and Technologies:

Genome Research Center (HKU)
<http://genome.hku.hk>

Bioinformatic Facilities (HKU)
<http://bioinfo.hku.hk>

Institute of Molecular Technology for Drug Discovery and Synthesis (HKU) led by Professor
<http://imt.chem.hku.hk>

Fred Hutchinson Cancer Research Center
 1100 Fairview Ave. N. PO Box 19024 Seattle, WA 98109
 ©2007 Fred Hutchinson Cancer Research Center, a non-profit organization.