

APPENDIX H.6

Report from Human Glycomics Initiative

Report (2009 – 2010) of Human Disease Glycomics/Proteomics Initiative (HGPI) activities

Hisashi Narimatsu, Chair of HGPI

We have performed the second pilot study as the HGPI activity, in which we analyzed O-glycan structure of three purified IgA1 samples by fifteen participating laboratories. The results summarized were published in *Molecular & Cellular Proteomics* as follows [ref 1]. The purpose of this study was an assessment of the current methodologies (i.e. mass spectrometric and chromatographic procedures) employed for O-glycomics analysis.

We are now performing the following two tasks under the theme of 'Glyco-Biomarker Discovery' in the third HGPI pilot study, that was initiated in the beginning of 2009. Task 1 is glycan structural analysis of both N- and O-glycans derived from the three different cancer cell lines, Hodgkin lymphoma cells, other lymphoma cells and neuroblastoma cells. Analyses of the global glycoprofile of these cancer cells is an easy way to gain knowledge about the types of N- and O-glycans attached to the proteins, and reflects the activity of the glycosylation machinery of the cell. This profile can be used to compare different types of samples to identify changes in glycosylation that occur in cancer. Task 2 is the identification of proteins which carry a special carbohydrate epitope. This task will be performed depending on the interest of each participant.

At the Symposium 3 of 9th HUPO World Congress in Sydney (September 20 2010, at 11:00-12:30 am), we present the Task 1 summary, which was glycan structural analysis of the three different cancer cells performed by the participating laboratories independently.

The results of Task 2 of the 3rd HGPI pilot study would be summarized at 10th HUPO World Congress in Geneva, Switzerland.

Publications

[1] Wada Y, Dell A, Haslam SM, Tissot B, Canis K, Azadi P, Bäckström M, Costello CE, Hansson GC, Hiki Y, Ishihara M, Ito H, Kakehi K, Karlsson N, Hayes CE, Kato K, Kawasaki N, Khoo KH, Kobayashi K, Kolarich D, Kondo A, Lebrilla C, Nakano M, Narimatsu H, Novak J, Novotny MV, Ohno E, Packer NH, Palaima E, Renfrow MB, Tajiri M, Thomsson KA, Yagi H, Yu SY, Taniguchi N., Comparison of Methods for Profiling O-glycosylation: HUPO Human Disease Glycomics/Proteome Initiative Multi-Institutional Study of IgA1. *Mol. Cell. Proteomics* 2010, 9, 719 - 727.

[2] Narimatsu, H., 8th HUPO World Congress: the Human Disease Glycomics/Proteomics Initiative (HGPI) Session 26 September 2009, Toronto, Canada. *Proteomics*. 2010, 10, 1899-902.

APPENDIX H.7

Report from Plasma Proteome Project

REPORT ON THE HUPO HUMAN PLASMA PROTEOME PROJECT (HPPP) FOR 2009-2010

Co-Chairs: Gil Omenn (USA), Ruedi Aebersold (Switzerland), Mark Baker (Australia)

The Plasma Proteome Project met jointly with the Cardiovascular Initiative in Toronto, as an experiment in combining the interests of two initiatives. Jennifer van Ejk, MingMing Ning, and Peipei Ping organized the CV component of the program, and Gil Omenn, Ruedi Aebersold, and Mark Baker organized the Plasma component.

Terry Farrah of the Institute for Systems Biology in Seattle presented a major update on the Human Plasma PeptideAtlas. Eric Deutsch of ISB, Henning Hermjakob and Lennart Martens of EBI, and Gil Omenn of Michigan (absent due to cardiac surgery) highlighted the ProteomeXchange scheme for submission of well-annotated datasets to EBI-PRIDE, Tranche-ProteomeCommons, ISB-PeptideAtlas, and NCBI-Peptidome. The HPPP is in the lead in demonstrating this process for all HUPO initiatives and for the wider proteomics community. Bernd Wollscheid of ETH Zurich presented the background of N-glycosite proteome analysis and plans for an MRM Atlas. An ABI QTRAP 4000 and an Agilent CHIP QTOF platform were used to generate SRM assays for >5000 human N-glycosites, corresponding to specific peptides selected from experimental data, Unipep, and PeptideAtlas databases. Michael Kuzyk of the University of Victoria - Genome BC Proteomics Centre in British Columbia, Canada, discussed an SRM/MRM study of 45 cardiovascular biomarker protein candidates analyzed in plasma from 60 patients with or without coronary artery disease; a set of 5 biomarkers differentiated the patients with and without CAD with up to 90% accuracy.

Discussion centered on these points: there is notable progress in enabling MS technologies and bioinformatics workflows, including studies of stroke, coronary heart disease, and myocardial dysfunction; the field is moving from discovery-driven workflows towards targeted experiments; and the SRM Atlas will be a good starting point for the community to download sets of transitions for proteins of interest in order to get a head start for their SRM measurements in clinical samples.

The plasma represents the final common pathway from organ proteomes as we seek to discover, confirm, validate, and utilize protein signatures and protein differential expression as biomarkers for diagnosis and prognosis. Extensive progress has been made during the past year in further updating the Human Plasma PeptideAtlas, with approximately 2000 protein IDs. A detailed set of thresholds has been devised at PeptideAtlas with increasing levels of stringency in terms decreasing likelihood of redundancy. Different thresholds are appropriate for comparisons with various external datasets. That work and the PeptideAtlas-2010 will be presented by Eric Deutsch in Sydney and is the subject of a manuscript expected to be submitted shortly.

As noted at the Toronto PPP/CVI Workshop due in part to the challenges of dynamic range and the dominance by highly abundant proteins in plasma, much attention has now been placed on targeted proteomics using selective or multiple reaction monitoring (SRM/MRM) of proteotypic tryptic peptides. An MRM Atlas is under development, led internationally by Ruedi Aebersold of ETH Zurich and Rob Moritz of ISB Seattle. Bernd Wollscheid of ETH will present major progress as part of the HPPP session on Wednesday 22 September of HUPO 2010 in Sydney. MRM adds the important dimension of absolute quantitation and avoids the dominance of abundant proteins.

As noted by the HUPO Human Proteome Project Working Group, the HPPP is a significant part of the foundation created by the HUPO Initiatives for the building of the Human Proteome Project.

There was one publication during 2009-2010 from the HPPP:

Omenn GS, Aebersold R, Paik YK. 7th HUPO World Congress of Proteomics: launching the second phase of the HUPO Plasma Proteome Project (PPP-2) 16-20 August 2008, Amsterdam, The Netherlands. *Proteomics* 2009; 9:4-6. PMID: 19053147.