



ICBC NEWSLETTER
INTERNATIONAL CANCER
BIOMARKER CONSORTIUM

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Dear ICBC Team Members:

Happy New Year to all. We are looking forward to another year of successful collaborations and progress within the Consortium.

It was wonderful to see so many of you in Singapore in early December. I was encouraged by the progress that has been made and the relationships being forged among teams. Presentations from the meeting will be posted on the secure CPAS website soon.

This newsletter includes a technology update from Dr. Daniel Liebler, Vanderbilt University Medical Center, on mapping and kinetic analysis of protein modifications.

CPAS Version 1.7 was released in December and contains several key enhancements. The team highlight for this issue is the Japan pancreas cancer team, led by Dr. Tesshi Yamada.

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. Please contact gcampaign@fhcrc.org if you would like us to organize a space at the Fred Hutchinson Cancer Research Center for a meeting.

Best regards,

Lee Hartwell
*President and Director
Fred Hutchinson Cancer Research Center*

SCIENTIFIC UPDATES

Mapping and kinetic analysis of protein modifications. New tools for characterizing protein modifications as potential biomarkers.

Dr. Daniel C. Liebler

Posttranslational modifications affect protein functions and also reflect the status of signaling pathways that are critical to the development of cancer and other disease. Protein damage by reactive electrophiles is a hallmark of oxidative stress, inflammation and chemical exposures that contribute to cancer and degenerative diseases (1). These endogenous electrophiles include reactive products of lipid, DNA and carbohydrate oxidation, which may modify protein nucleophiles to produce a variety of adducts, the majority of which have yet to be well characterized.

Analysis of protein modifications is best accomplished by shotgun proteome analyses, which generate MS-MS spectra that encode the sequences of modified peptides and the masses and sequence positions of modifications.

In cases where the positional specificity and mass of modifications are known (e.g., phosphorylation, which adds +80 Da to S, T and Y residues) database searches of MS-MS spectra with the specified modifications results in identification of modified sequences. However, for modifications arising from reactive chemical species (e.g., lipid oxidation products and glycation products), the mass and sequence specificity are more difficult to predict and the use of standard database search algorithms produces a high rate of false-positive identifications. The P-Mod algorithm (2) and similar software tools enable identification of MS-MS spectra corresponding to modified peptide sequences, map the modifications to specific sequences and estimate the probability that the matches are nonrandom assignments. A specific advantage of P-Mod is that the masses of sequence specificities of the modifications need not be anticipated or specified in the analysis. Thus, this approach is ideas for discovery of unanticipated, novel modifications.

In studies directed at predicting potential biomarkers of oxidative stress in vivo, we used LC-MS-MS analyses and P-Mod to map 10 sites of covalent modification of human serum albumin (HSA) by the reactive lipid oxidation product 4-hydroxynonenal (HNE) (3). To measure the rates or reaction at six His and Lys residues in the HSA protein, we applied a stable isotope tagging strategy which employs N-terminal tagging of peptides with heavy ($^{13}\text{C}_6$ -PIC) and light ($^{12}\text{C}_6$ -PIC) labeled phenylisocyanate (PIC) (4). Peptides from the last time point in the experiment (which corresponds to the highest adduct levels) are tagged with $^{13}\text{C}_6$ -PIC, whereas peptides from the other time points are tagged with the $^{12}\text{C}_6$ -PIC (Figure 1). Targeted LC-MS-MS analysis the light and heavy PIC labeled adducts enable selective detection of each. Plots of the ratios of light:heavy PIC-labeled adduct yield measurements of kobs values, which indicate relative reaction rates for all of the detectable sites. This approach allows simultaneous quantitative comparison of all adduction reactions in the same protein.

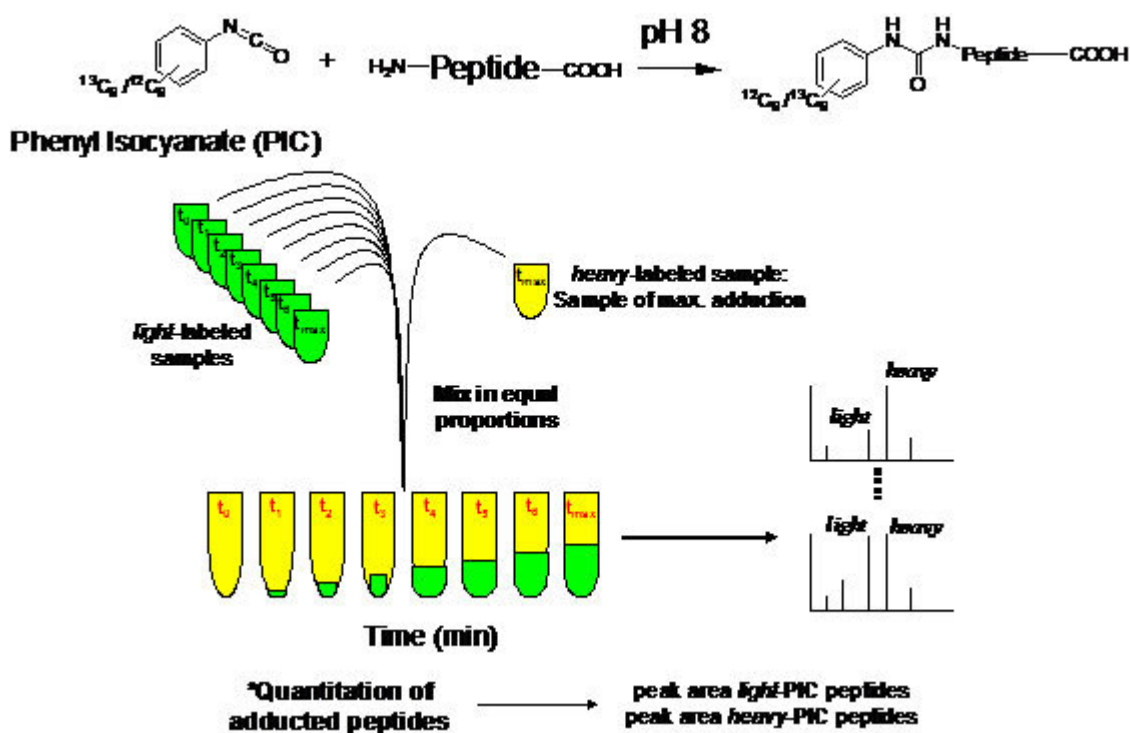


Figure 1. Stable isotope tagging and relative quantitation with PIC.

Analysis of the most reactive sites in the context of the HSA protein structure provided interesting insights into factors that dictate reactivity and selectivity in covalent adduction (3). Solvent-exposed Lys and His residues displayed reactivities that varied over about two orders of magnitude (Figure 2).

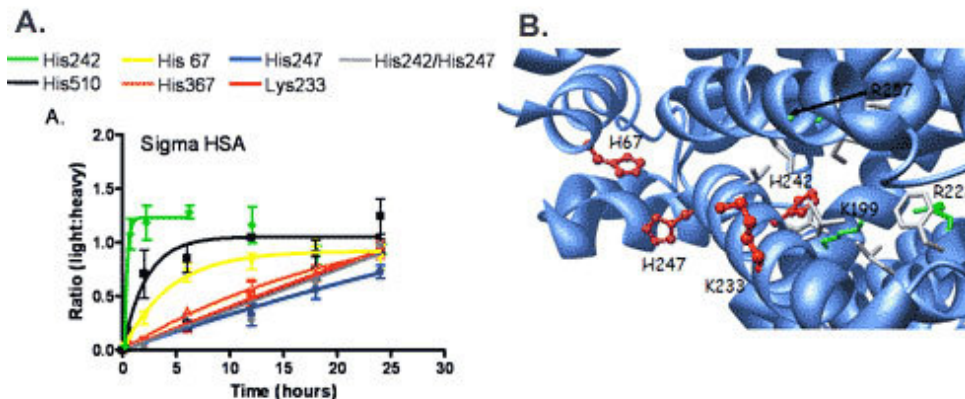


Figure 2. A. Kinetic profiles for adduction of HSA at six reaction sites (H242/H247 contains two histidine adducts on the same tryptic peptide). B. Locations of adducted His and Lys residues in the IIA subdomain of HSA. The most reactive target, His242, lies in a hydrophobic pocket and the pK of the imidazole nucleophile is predicted to be reduced by desolvation effects and neighboring basic residues Lys199 and Arg222. (Reproduced with permission from reference 3. Copyright (2006) American Chemical Society.)

However, the most reactive target was His242, which resides in a hydrophobic binding pocket known to bind fatty acids and several drugs. Moreover, the predicted pKa (<2.0) of His242 account for its high reactivity toward HNE. His242 adducts may be preferred products of adduction by lipophilic electrophiles and may comprise a family of biomarkers for oxidative stress.

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3. Szapacs, M. E., Riggins, J. N., Zimmerman, L. J., and Liebler, D. C. (2006) Covalent adduction of human serum albumin by 4-hydroxy-2-nonenal: kinetic analysis of competing alkylation reactions. *Biochemistry* 45, 10521-8.
4. Mason, D. E., and Liebler, D. C. (2003) Quantitative analysis of modified proteins by LC-MS-MS of peptides labeled with phenyl isocyanate. *J.Proteome Res.* 2, 265-272.

CPAS 1.7 is now available! CPAS Release 1.7 includes many new features. A few key enhancements are listed below.

- MS2 run comparisons using ProteinProphet data
- Mascot support
- Improved sample information handling
- Discussion board enhancements
- Reporting improvements

To learn more about CPAS Release 1.7 or to download a copy, please visit <https://cpas.fhcr.org/Project/home/home.view>.

RECENT PUBLICATIONS

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COLLABORATIVE SOFTWARE

None at this time.

ADMINISTRATIVE UPDATES

Minutes from the December ICBC meeting are nearly finalized and will be distributed when complete.

BIOMARKER TEAM SPOTLIGHT

Pancreas — National Cancer Center Research Institute (Coordinating Center)

Project Title:	Improving Early Detection of Pancreatic Cancer with a Blood Test
Cancer Site(s):	Pancreas
Principal Investigator(s):	Tesshi YAMADA, M.D., Ph.D.
Participating Institutions:	National Cancer Center Research Institute (Coordinating Center) Tokyo Medical University Clinical Proteome Center
Mouse Model(s):	Under consideration
Clinical Samples:	Plasma/sera from Jichi Medical School Hospital Tokyo Medical University Hospital National Cancer Center Research Hospital Osaka Medical Center for Cancer and Cardiovascular Diseases Fukuoka University Hospital
Technical Approaches:	Glycopeptide enrichment Surface Enhanced Laser Desorption/Ionization hybrid Quadrupole Time-of-Flight Mass Spectrometry (SELDI-QqTOF-MS) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-MS) Two Dimensional Difference Gel Electrophoresis (2D-DIGE) Protein/peptide quantitation using isotope-coded affinity tags (ICAT) Two Dimensional Image Converted Analysis of nano-flow Liquid chromatography and Mass Spectrometry (2D-ICAL-MS) Two-dimensional microflow liquid chromatography/tandem mass spectrometry (2-D microLC-MS/MS)

Brief Description of Project:

Pancreatic cancer is the fifth leading cause of cancer-related mortality in Japan and was responsible for 22260 deaths in Japan in 2004. The number has increased about 2.5 fold over the last 20 years, and will continue to increase in the future. Since the clinical manifestations of pancreatic cancer, except obstructive jaundice, are often not apparent until the advanced stages of the disease, and the anatomical location of the pancreas deep in the abdomen makes physical and ultrasonic detection of pancreatic cancer difficult, about 95% of all cases are diagnosed in stage III or IV, and the 5-year survival rate of pancreatic cancer patients is the lowest among patients with common solid tumors. Early detection by mass screening seems to be one of the most feasible strategies for improving the outcome of pancreatic cancer patients. Since the 5-year survival rate of pancreatic cancer patients with stage I, II, III, IVa, and IVb disease has been reported to be 59%, 51%, 26%, 12%, and 3%, respectively, detection of stage I or II disease would significantly improve overall patient survival.

Mass screening by computerized tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET) may not be cost-effective because of the relatively low incidence of pancreatic cancer, and the long-term safety of these modalities has not been established. Thus, the development of a new diagnostic modality that enables the early detection of pancreatic cancer in a safe/non-invasive and cost-effective way is needed. Human blood serum and plasma contain a large variety of proteins, and their relative abundance and modification may precisely reflect the disease status of organs and tissues. Recent advances in MS-based proteomic technologies coupled with bioinformatics may revolutionize medical diagnosis and cancer screening. The purpose of the team is to identify new diagnostic biomarkers of pancreatic cancer by comprehensive serum/plasma protein profiling. This team is composed of clinicians and scientists specialized in proteomics and bioinformatics. A large number of serum/plasma samples will be prospectively collected from participating institutions under the same protocol. Protein profiling will be performed by using various cutting-edge technologies, such as SELDI-QqTOF-MS, MALDI-MS, 2D-DIGE, ICAT, 2D-ICAL-MS, and 2-D microLC-MS/MS.

This team is supported by the grant "Third Term Comprehensive Control Research for Cancer" from the Ministry of Health, Labor and Welfare of Japan.

Team Members and Expertise:

- **Kazufumi HONDA, D.D.s., Ph.D. (National Cancer Center Research Institute)** Section Head of National Cancer Center Research Institute Cancer Proteomics Project Bioinformatics and Proteomics (SELDI-QqTOF-MS and MALDI-MS)
- **Tatsuya IOKA, M.D. (Osaka Medical Center for Cancer and Cardiovascular Diseases)** Chief Physician of Osaka Medical Center for Cancer and Cardiovascular Diseases Chemotherapy and Mass Survey Provision of Clinical Samples
- **Tadashi KONDO, M.D., Ph.D. (National Cancer Center Research Institute)** Section Head and Project Leader of National Cancer Center Research Institute Proteome Bioinformatics Project Bioinformatics and Proteomics (2D DIGE)
- **Hideo NAGAI, M.D., Ph.D. (Jichi Medical School)** Professor of Jichi Medical School General and Pancreatic Surgery Provision of Clinical Samples
- **Toshihide NISHIMURA, Ph.D. (Tokyo Medical University)** Professor of Tokyo Medical University Proteomics (2-D microLC-MS/MS)
- **Takuji OKUSAKA, M.D., Ph.D. (National Cancer Center Hospital)** Chief Physician of National Cancer Center Hospital Hepatobiliary and Pancreatic Oncology Provision of Clinical Samples
- **Masaya ONO, M.D., Ph.D. (National Cancer Center Research Institute)** Section Head of National Cancer Center Research Institute Cancer Proteomics Project Proteomics (2D-ICAL-MS)
- **Miki SHITASHIGE, Ph.D. (National Cancer Center Research Institute)** Staff Scientist of National Cancer Center Research Institute Cancer Proteomics Project Proteomics (ICAT)
- **Akihiko TSUCHIDA, M.D., Ph.D. (Tokyo Medical University)** Associate Professor of Tokyo Medical University Hepatobiliary and Pancreatic Surgery Provision of Clinical Samples
- **Tesshi YAMADA, M.D., Ph.D. (National Cancer Center Research Institute-Biomarker Team PI)** [tyamada@ncc.go.jp] Chief of Chemotherapy Division and Project Leader of Cancer Proteomics Project Bioinformatics
- **Yohichi YASUNAMI, M.D., Ph.D. (Fukuoka University)** Associate Professor of Fukuoka University Gastrointestinal Surgery Provision of Clinical Samples