PCT-related Poster Abstracts from ASMS 2014

A rapid, data independent acquisition method for population-scale proteome barcoding using PCT-SWAT

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Introduction

Population-scale proteomic analysis is essential for biomarker studies and, more broadly, for personalized and precision medicine. A barcode is the representation of data that can be used to rapidly identifying a unique object. Biological samples from different species are being catalogued using DNA barcodes, however, DNA is not the ideal material to distinguish samples from the same species. We introduce here the concept "proteome barcoding" as a new mass spectrometry based methodology for producing representative proteomic BGI DATA for population-scale biological samples. The methodology has 3 key features: 1) fast digitization of proteome; 2) minimal sample consumption and, 3) reproducible and comprehensive BIG proteomic DATA.

Methods

To implement "proteome barcoding", we developed a method for the fast mass spectrometric conversion of small amounts of sample into a single, permanent digital file representing the quantitative proteome of the sample. The thus generated proteome maps can then be perpetually analyzed, compared and mined in silico. The method combines pressure cycling technology (PCT) and SWATH mass spectrometry. The resultant data were analyzed using software tools including OpenSWATH.

Preliminary Results/Abstracts

We demonstrated "proteome barcoding" from three case studies. 1: The PCT-SWATH method was applied to study 18 biopsy samples collected from renal cell carcinoma patients. Clinical tissue biopsy samples of 1mg wet weight were successfully converted into SWATH-MS fragment ion maps within one working day with 6 samples being processed with a time window of 36 hours. The data further show that from the resulting SWATH-MS maps more than 2000 proteins could be detected and quantified with a high degree of reproducibility and that the resulting proteome maps clearly separated tumorous kidney tissues from their control counterparts and also separated different histomorphological kidney cancer subtypes. 2: Quantification of 60 different human proteomes (NCI60 cells) with replicates were accomplished within 30 working days. Over 40 000 peptides from over 4000 Swissprot proteins were quantified across the samples. Protein complexes were quantified across 60 proteomes. We evaluated the drug sensitivity predictive power of the SWATH data sets compared to other available genomic and proteomic data sets. 3: Quantification of 30 punches from different regions of prostate cancer tissues permits for the first time quantitative analysis of tumor heterogeneity at proteomic level. Stable and variable proteins were characterized.

Novel Aspect

Here we move proteomics to population-scale analysis. We have coined a new concept “proteome barcoding”

Variables Affecting the Quality of Digestion-based Protein Quantification: Implications of Enzyme Kinetics on Clinical Measurements

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Introduction

It has recently been demonstrated rapid proteolysis is critical to achieving accurate quantitation in protein cleavage isotope-dilution mass spectrometry (PC-IDMS) assays when the surrogate/target peptide is labile. Given the inherent implication to clinical measurements, we have performed kinetic studies of trypsin-based proteolysis for 8 potential surrogate peptides of Thyroglobulin, a tumor marker which is often quantified by PC-IDMS in clinical settings due to interference in immunometric measurements. Multiple factors purported to increase the rate of proteolysis were evaluated to determine their impact on quantitative accuracy, including the influence of competitive interference on the rate of proteolysis. Cleavable stable isotope-labeled (SIL) peptides were tested for their ability to correct for variance in the rate and efficiency of proteolysis across various conditions.

Methods

Time course analysis of various digestion protocols were conducted on pooled serum spiked with exogenous thyroglobulin. Thyroglobulin was spiked at supraphysiological levels in order to facilitate downstream LC-MS/MS measurements without further rigorous fractionation/enrichment. Immediately preceding the addition of trypsin, specimens were spiked with a cocktail of 8 cleavable-SIL peptides, each containing one labeled amino acid, and each corresponding to a native peptide sequence (including proteolytic sites for trypsin digestion). At defined time points, digestions were quenched with acid. Subsequently, recovery of native and SIL proteolysis products was determined by adding a second cocktail of SIL peptide standards, each containing 2 labeled amino acids, and each matching the sequence of the fully cleaved peptide products.

Preliminary Results/Abstract

It was recently revealed peptide degradation during PC-IDMS could induce a positive bias when the rate of proteolysis is slow (Muddiman & co-workers, Mol. Cel. Proteomics, 2012). Consequently, various methodologies were tested herein to increase the rate of proteolysis for 8 potential surrogate peptides of Thyroglobulin. Preliminary results show increasing the concentration of trypsin by an order of magnitude can increase the rate of digestion 2 to 10-fold. For one peptide, this also increased the rate of peptide degradation ~3-fold, presumably due to non-specific cleavage. To that end, various forms of modified and immobilized trypsin are currently being evaluated to determine if these high-grade trypsins can improve the rate of the proteolysis further and/or decrease the rate of peptide degradation.

SIL peptides incorporating cleavage sites were also tested as internal standards to see if they could mimic the rate of protein proteolysis and, thereby, minimize decay-induced bias. For 7 of 8 cleavable peptide internal standards, the rate of production/digestion was determined to be >2-fold faster than the rate for their native counterparts. Indeed, 7 of 8 cSIL peptides were observed to reach complete digestion within 15 minutes - the shortest time tested.

It is hypothesized the rate of proteolysis will be dependent upon the amount of competitive inhibition in a sample - in other words, the total protein concentration - and kinetic studies at various total protein concentrations are underway to study this possibility. This has implications in clinical PC-IDMS measurements where serum total protein levels can vary and where normalizing each sample

prior to digestion based on total protein content is impractical. Moreover, this may impact the utilization of calibrator matrices where protein calibrators could experience a different rate of proteolysis than endogenous proteins, not to mention the use of recombinant protein calibrators that digest at different rates than endogenous proteins.

Novel Aspect

These studies demonstrate utilizing stable surrogate peptides and increasing rates of proteolysis are paramount for achieving absolute accuracy by PC-IDMS.

Serovar and strain level bacterial differentiation capabilities for 36 closely related outbreak strains by intact protein LCMS

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Introduction

ESI-LCMS analysis of intact protein bacterial lysates generates unique protein expression profiles for bacterial differentiation. For traceback of food contamination serovar level differentiation is necessary and strain level typing ideal. Most bacterial protein LCMS efforts have been limited to comparison between two samples. We present LCMS intact protein expression profile results from participation in a semi-blinded study of 36 Salmonella isolates originating from foodborne outbreaks. Study creators established sample relatedness at the serotype, PFGE, and whole genome sequence levels. LCMS generated intact protein expression profiles were used to divide isolates into resolvable clades. These clades were compared to those generated from the above mentioned genomic techniques.

Methods

Subtyping method outbreak validation study and samples were provided by the FDA-CFSAN. For LCMS analysis, Salmonella isolates were cultured over night on TSA plates, harvested, and extracted in 50:49:1 acetonitrile, water, formic acid using the Barocycler Pressure Cycling Technology system (Pressure BioSciences Inc.). On-line separation of proteins was performed with a C8 Kinetex (Phenomenex) column coupled to an Aquity UPLC (Waters) and Q-ToF Premier (Waters) mass spectrometer. Each of 36 isolates was run in triplicate. All data was deconvoluted and binned to accurate mass, retention time, and intensity expression profiles with ProteinTrawler (BioAnalyte) software. Intact protein expression profiles were aligned and statistically filtered using in-house developed software PathProt. HCA and PCA was performed with Piroutte software (InfoMetrix).

Preliminary Results/Abstract

The 36 isolates were run in triplicate by ESI-LCMS. Deconvolution of LCMS runs generated binned accurate mass, retention time, and abundance profiles. Visual comparison of intact protein mass profiles easily defined serovar specific marker masses. The correct serovar was assigned to all 36 isolates. Sub-serovar level clades could also be defined based on other unique masses. Visual analysis of mass shifts across isolates defined clades at a level comparable to pulsed field gel electrophoresis (PFGE), the current bacterial subtyping gold standard. Efforts were made to automate cluster analysis and differentiation. Replicate runs were normalized and filtered based on summed total intensity, binned based on mass and retention time alignment and filtered with a RSD cut off of 100%. The resultant list representing masses appearing in two out of three replicates was aligned with the filtered mass lists from all other isolates. The final output was a matrix consisting of binned masses and abundances across 36 isolates. Matrices were transferred to chemometrics software for hierarchical clustering analysis and principle component analysis. Both methods did a reasonable job defining three of four serovar groups but neither could consistently separate a fourth from a closely related group. Matrices were tested as abundance and presence/absence based as well as filtered at a range of stringencies. The inability of off the shelf software tools to define differentiable masses that are clearly present in the midst of a much larger number of non-changing masses strongly suggests that a primary bottle neck for LCMS bacterial profiling is development of statistical analysis software that can distinguish unique differentiable masses in the midst of unchanging masses at least as well as visual analysis. Outbreak samples will also be analyzed by MALDI-MS to assess if the decrease in the number of masses detected improves the success of chemometric analysis tools.

Novel Aspects

Serovar and strain level bacterial differentiation capabilities of intact protein LCMS for 36 closely related Salmonella outbreak strains.

Large-Scale Quantitative Proteomic/Metaproteomic Platform Discovers Target Pathways and Promising Biomarkers of COPD-associated Lung Cancer

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Introduction

Chronic Obstructive Pulmonary Disease (COPD), independent of smoking, is an emerging risk factor for lung cancer. Unfortunately, little has been accomplished to understand this link, and we lack data on potential therapeutic targets and biomarkers indicative of COPD-associated lung cancer. Furthermore, although appreciated, we lack understanding of the contribution of the lung microbiome on these conditions. To fill this gap, we have developed a comprehensive analysis platform employing innovative clinical sample preparation, iTRAQ labeling, and computational analysis using the flexible Galaxy-P platform. This platform enables comprehensive quantitative proteomic/metaproteomic analysis in tens of patient samples. We have generated novel data on disease-associated host proteins and microbiome contributions, identifying affected molecular pathways and promising biomarkers of COPD-associated lung cancer.

Methods

We obtained 80 flash frozen lung tissue samples with various degrees of COPD severity with/without lung cancer. Samples were homogenized using a bead mill homogenizer at maximum setting. Cells were lysed and membranes dissociated via hydrostatic pressure cycling (Barocycler). Following trypsin digestion the samples were labeled with 8-plex iTRAQ reagents. Following high-pH and reversed-phase separation LC-MS analysis was performed using an Orbitrap Velos. Raw files obtained directly from the Orbitrap Velos Mass Spectrometer were imported into Galaxy-P, an extension of the genomics-based Galaxy framework for proteomic analysis and management. A ProteinPilot 4.5 search was performed with a custom database containing human and

microbial proteins. Workflows within Galaxy-P were used for data processing for proteomic and metaproteomic analysis (http://z.umn.edu/8plexmp and http://z.umn.edu/dp2blast2megan5).

Preliminary Results/Abstract

Extensive method development, including optimizing lung tissue sample processing followed by offline fractionation, enabled maximized protein identification and quantification. To compare data across multiple 8-plex iTRAQ experiments, a pooled standard was developed. An equal amount of protein from 80 samples was homogenized and aliquoted. Each 8-plex run contain a pooled standard (119 and 121 iTRAQ channel), with six blinded patient samples in the other channels. The two standards serve as an internal control for each run providing a global false discovery rate (FDR) for protein quantification. We have observed an average of 1900 high-confidence protein identifications through 10-iTRAQ runs analyzing 60 patient samples. Using the Galaxy-P environment, a sequence database search was performed with a custom database, containing host human proteins and microbial protein sequences. The resulting single summary data sheet, peptide list, and protein list were then imported into the Protein Pilot™ Descriptive Statistics Template (PDST). PDST analysis provides identification and quantification metrics such as - characterization of digestion quality, modification frequencies, and computation of quantitative FDR for differential expression. Using customizable Galaxy-P workflows, we identified and quantified human proteins as well as microbial peptides. Microbial peptides belonging to Actinomyces viscosus and Pseudomonas syringae species were identified. Functional and quantitative analysis of microbial protein expression is being carried out to determine microbial profiles that are associated with lung cancer development. For host human proteins, those quantified with high confidence were further analyzed via Ingenuity Pathways Analyses (IPA) software. Several molecular pathways including inflammation and tissue remodeling are up-regulated in COPD and lung cancer patients. Interestingly, known cancer markers increase abundance with worsening COPD, and continue this trend with patients transitioning to lung cancer. Collectively, our results have defined new pathways of COPD-associated lung cancer for possible drug intervention, and identified numerous promising candidate diagnostic biomarkers.

Novel Aspects

A unique platform enabling comprehensive identification of molecular pathways, biomarkers, and microbiome contributed proteins to COPD-associated lung cancer

Rapid Identification of Beta-carboline Hallucinogens: Harmine and Harmaline, by Pressure Cycling Technology (PCT) and DMS-MS

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Introduction

A comparative analysis was performed using GC/MS and Differential Mobility Spectrometry - Mass Spectrometry (DMS-MS) for the analysis of Peganum harmala seeds. Ions corresponding to the ?-carboline hallucinogens, harmine and harmaline, which differ by one level of saturation in their pyridine rings, were separated and detected from a single seed prepared for analysis utilizing pressure cycling technology. A direct comparison between GC/MS and DMS-MS is shown in an effort to evaluate DMS as a rapid analysis method for trace drugs of abuse from plant-based matrices. DMS prior to mass analysis allows an analyst to separate a population of electrosprayed ions and has demonstrated promising research findings for the high throughput analysis of forensically relevant and structurally similar drugs of abuse.

Methods

The goal of this research was to develop a sensitive and expeditious analytical scheme for the characterization of suspected P. harmala seeds. In an effort to illustrate a faster and more efficient extraction method, harmine and harmaline were extracted from seeds using an adapted method based on pressure cycling technology (PCT). In order to evaluate the feasibility of PCT, an average mass of 3 mg (single seed) of dried, crushed seeds in 100 ?L of methanol were extracted using the pressure cycler at 35,000 psi with a total run time of approximately 20 minutes. Following extraction, a direct comparison between GC/MS, commonly utilized in forensic analysis, and DMS/MS was performed.

Preliminary Results/Abstract

Several analytical methods with various sensitivity and selectivity have been utilized for the identification and analysis of these two alkaloids. Such methods include HPLC in combination with UV, chemiluminescence, fluorometry, MS, and GC-MS. Although GC separations offer many advantages, it often requires chemical derivatization to improve detection and separation of non-volatile, polar or thermally labile compounds, which results in lengthy sample preparation. Additionally, chromatographic separations remain time-consuming and there is an ongoing interest in higher throughput and more efficient technologies for larger-scale analysis of drugs. Comparative extractions between traditional liquid-liquid extractions of pulverized seeds and a PCT-based methodology determined that the PCT extraction protocol resulted in an average yield of 0.3 mg of harmine and harmaline from a single 3 mg seed; approximately 10% by weight drug and nearly 100% extraction efficiency based on reported literature values.

The use of an appropriate transport-gas modifier improves ion selectivity and separation and is an essential consideration in the development of DMS-based separation methods. Harmine and harmaline, which differ by only one level of saturation in the pyridine ring fused to the indole ring of the ?-carboline, were effectively separated by the use of ethyl acetate as a transport-gas modifier. The identifications of harmine and harmaline were confirmed by MS-MS experiments relying on transitions from 213 to 198 for harmine and from 215 to 200 and 174 for harmaline, respectively. The combination of ion filtration, in tandem with a mass spectrometer allowed for the rapid separation (less than 1 minute) of the structurally similar hallucinogenic compounds. The high selectivity afforded by the orthogonal coupling of these two analytical techniques provides the conditions for enhancing the detection of targeted drugs from plant-based matrices. By comparison, the GC/MS method required as many as 20-minutes to accomplish separation of the target compounds.

Novel Aspects

Enhanced sensitivity by PCT followed by rapid separation and analysis by DMS-MS for structurally similar Beta-carboline hallucinogens: harmine and Harmaline.

Alternation of Glycans Site Specificity in Patients with Liver Diseases

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Introduction

Glycosylation dramatically influence biochemical properties of proteins. It is known that glycans structure changes under non-physiological conditions such as disease or inflammation. These changes have diagnostic potential and could be used as disease biomarkers. The maturation of glycans is controlled not only by the enzyme activities of glycosidases and glycosyltransferases, but also by the protein structure. Here we present data showing the site specific glycan alternation of several serum proteins isolated from patients with liver diseases.

Methods

Sera from patients with hepatocellular carcinoma (HCC), colorectal cancer and colorectal cancer with metastases to the liver were used for isolation of haptoglobin. Other glycoproteins including hemopexin, complement factor H and kininogen-1 were isolated from individual HCC patient. For determination of glycan site specificity, proteins were digested by trypsin, treated with exoglycosidases and analyzed by nanoLC connected to either Q-Tof or FT-ICR mass spectrometer. Glycopeptides were identified by GlycoPeptideSearch software and validated by manual data inspection. For structural characterization of altered glycoforms, isolated glycoproteins were treated with PNGaseF. Released glycans were permethylated following standard protocol and analyzed by multi-stage CID fragmentation.

Preliminary Results/Abstract

The case-study of HCC patient compares site specific glycoforms of four proteins including haptoglobin, complement factor H, kininogen-1 and hemopexin. Exoglycosidases-assisted LC-MSMS analysis confirms high degree of fucosylation of some of the proteins but the microheterogeneity is protein and site-specific. Kininogen-1 and haptoglobin carry up to six fucoses per glycan while hemopexin contains glycoforms with a maximum of three fucoses per glycan at one specific sites and complement factor H carries at most two fucoses at any given site. MSn analysis of permethylated detached glycans confirms presence of LeY glycoforms on haptoglobin which cannot be detected in hemopexin or complement factor H; all three carry LeH glycoforms but core fucosylation is detectable only on hemopexin and complement factor H. The comparison study between patients with HCC, colorectal cancer and colorectal cancer with metastases to the liver shows differences in expression of multi-fucosylated glycoforms in haptoglobin. The maximum of three fucoses per glycan were observed. These glycoforms increased significantly in patients with HCC compared to healthy individuals and patients with colorectal cancer.

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Novel Aspects

Glycan site specificity is protein and peptide specific.

Mass spectrometry-based proteomics of human induced pluripotent stem cells (hiPSC) cultured in suboptimal culture conditions

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Introduction

Human induced pluripotent stem cells (hiPSCs) offer unprecedented potential for drug discovery, toxicology, regenerative medicine, and disease research. The quality of the stem cells has a great impact on how the cells could be utilized in future applications. Suboptimal culture conditions, such as prolonged culturing beyond confluency may result in metabolic and phenotypic changes in cells that may pose safety risks or produce inferior quality of products. Currently there is no effective technique available to monitor global quality of hiPSC in cell culture. Here we applied comprehensive qualitative and quantitative proteomics to monitor proteome changes during the course of prolonged culture (suboptimal culture conditions) and aimed to identify a panel of proteins that could predict the quality of cells.

Methods

To build a database of specific molecular markers indicative of suboptimal cell product quality characteristics, we performed mass spectrometry (MS)-based proteome characterization of hiPSCs of different somatic origin: CD34+ cells circulating in peripheral blood and fibroblasts of healthy donors. Each iPSC line was established using excisable polycistronic Lentivirus vector 'STEMCCA-loxP' (Millipore) and cultured in albumin-free E8 medium up to 80% confluency (standard culture condition: control). Each cell line was then overgrown for 1, 3, or 5 days changing the medium daily (suboptimal culture condition: mistreated). The cells were then collected, lysed, and the proteins were extracted for MS analysis. Global proteome analysis was performed by 2D-LC/HDMSE using UPLC and Synapt G2 mass spectrometer (Waters Corporation, Milford, MA).

Preliminary Results/Abstracts

On average about 1650 proteins were confidently quantified from both cell lines. We identified 30 proteins that were up-regulated in both overgrown hiPSCs derived from fibroblasts and CD34+ hematopoietic cells. Some of the up-regulated proteins, such as galectin-1, galectin-3-binding protein, insulin-like growth factor 2 mRNA-binding protein 2 (IMP-2) are known to be involved in apoptosis. Several of the up-regulated proteins were lysosomal proteases, which are also involved in the progression of apoptosis. In addition, we identified 12 proteins that are down-regulated in the overgrown cells. One of these proteins, Thioredoxin, is known to play an inhibitory role to cell death. The expression level of several known pluripotent markers, such as PODXL (TRA-1-60/TRA-1-81), NEST, TUBB3, LIN28A, DNMT3B didn't change significantly over prolonged culturing. Differentially expressed proteins with a significant fold change will be further verified by Western blot and other methods. Ingenuity Pathway Analysis of the proteins differentially expressed between the control and overgrown cells revealed that the top 5 biological functions commonly up-regulated in overgrown samples of both hiPSCs were cell death and survival, lipid metabolism, molecular transport, small molecule

biochemistry, and nucleic acid metabolism. The proteomic approach developed for hiPSC characterization has the potential to be used for other specific applications relative to expansion and differentiation of various stem cell types.

Novel Aspects

Identification of a panel of proteins that could potentially be used for prediction of cell quality.

High Pressure-Assisted Extraction for the Improved Proteomic Analysis of FFPE Tissue

Authors

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Introduction

Mass Spec-based proteomics hold great promise for developing knowledge of the molecular characteristics of disease. Formaldehyde-fixed, paraffin-embedded (FFPE) tissue repositories represent an invaluable resource for the retrospective study of disease progression and response to therapy. However, the analysis of FFPE tissues by proteomic methods has been hampered by formaldehyde-induced protein adducts and cross-links. Here, we demonstrate the use of heat augmented with high hydrostatic pressure (40,000 psi) as a novel method for the recovery of intact proteins from FFPE mouse liver.

Methods

FFPE mouse liver sections were cleared of paraffin and homogenized in 50 mM Tris-HCl, pH 7, 2% SDS or 100 mM Tris-HCl, pH 8, 100 mM DTT, 4% SDS. The tissue was incubated 100°C for 30 min followed by 80°C for 2 h at 1 atmosphere, or at 40,000 psi. Extracts from matched fresh tissue and the FFPE tissue were separated by SDS-PAGE, and the in-gel tryptic digests of each sample were analyzed by LC-MS/MS.

Preliminary Results/Abstracts

When FFPE mouse liver was extracted using heat and elevated pressure, there was a 4-fold increase in protein extraction efficiency and up to a 30-fold increase in the number of non-redundant proteins identified by mass spectrometry, compared to matched tissue extracted with heat alone. More importantly, the number of non-redundant proteins identified in the FFPE tissue was nearly identical to that of the corresponding frozen tissue.

Novel Aspects

This study shows that high pressure treatment improves the quality and yield of proteins extracted from archival tissue.

Elevated Pressure Improves the Extraction and Identification of Proteins Recovered from Formalin-Fixed, Paraffin-Embedded Tissue Surrogates

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Introduction

Proteomic studies of formalin-fixed paraffin-embedded (FFPE) tissues have been hampered by the inability to extract proteins from archival tissue in a form suitable for analysis by 2-D gel electrophoresis or mass spectrometry. This inability arises from the difficulty of reversing formaldehyde-induced protein adducts and cross-links within FFPE tissues. We previously reported the use of elevated hydrostatic pressure as a method for efficient protein recovery from a hen egg-white lysozyme tissue surrogate, a model system developed to study formalin fixation and histochemical processing.

Methods

To model FFPE tissue, a 150 mg/mL solution of lysozyme or a protein mixture consisting of lysozyme, carbonic anhydrase, ribonuclease A, BSA, and myoglobin (55:15:15:10:5 w/w) was mixed with an equal volume of 20% phosphate-buffered formalin, and paraffin-embedded according to standard histological protocols. The tissue surrogates were resuspended in Tris-HCl, with 2% (w/v) SDS, and incubated at 100 °C for 30 min followed by 80°C for 2 h under atmospheric pressure or elevated pressure 40,000 psi. The FFPE tissue surrogate extracts were analyzed by RPLC coupled in-line with an Agilent 6340 ion trap mass spectrometer. The effects of elevated pressure on the rate of reversal of formaldehyde-induced protein cross-links and aggregate size were investigated by SDS-PAGE and dynamic light scattering.

Preliminary Results/Abstracts

In this study, we demonstrate the utility of elevated hydrostatic pressure as a method for efficient protein recovery from a complex multi-protein FFPE tissue surrogate comprised of hen egg-white lysozyme, bovine carbonic anhydrase, bovine ribonuclease A, bovine serum albumin, and equine myoglobin (55:15:15:10:5 wt%). Mass spectrometry of the FFPE tissue surrogates retrieved under elevated pressure showed that both the low and high-abundance proteins were identified with sequence coverage comparable to that of the surrogate mixture prior to formaldehyde treatment. In contrast, non-pressure-extracted tissue surrogate samples yielded few positive and many false peptide identifications. Analysis of the raw MS data also revealed several differences in the quality of the tissue surrogate extracts. Since the tissue surrogates consisted of a defined set of proteins, it was possible to calculate the average false protein identification rate for each sample. The false identification rates for the pressure extracted multi-protein surrogate samples were 5.7% (pH 8) and 7.8% (pH4), which was comparable to the precursor mixture, with a false identification percentage of 3.3%. The false identification rate for the non-pressure extracted tissue surrogates was 42% to 100%. There were also fewer total peptides identified in the non-pressure treated samples, approximately 10% the number identified for the pressure treated and precursor samples Studies with soluble formalin-treated bovine ribonuclease A demonstrated that pressure modestly inhibited the rate of reversal (hydrolysis) of formaldehyde-induced protein cross-links. Dynamic light scattering studies suggest that elevated hydrostatic pressure and heat facilitate the recovery of proteins free of formaldehyde adducts and cross-links by promoting protein unfolding and hydration with a concomitant reduction in the average size of the protein aggregates.

Novel Aspects

These mechanistic studies demonstrate that elevated pressure treatment is a promising approach for improving proteomic analysis of FFPE tissue.