demonstrate the utility of this integrative approach in mechanistically probing antiviral immune signaling.

Mon Talk 10:55-11:10 am: Proteomic analysis reveals novel hydroxyproline-dependent cellular pathways in cancer cells

Luke Erber; Tong Zhou; Yue Chen
University of Minnesota, Minneapolis, MN

Proline hydroxylation (Hyp) is a critical cellular mechanism regulating oxygen-response pathways in tumor initiation and progression. We developed a proteomics strategy based on immunoaffinity enrichment to identify novel hydroxyproline-modified substrates. By integrating antibody based hydroxyproline enrichment with exhaustive HPLC-mass spectrometry analysis, we identified 562 proline hydroxylation sites on 272 protein substrates in HeLa cells. These identifications include many well-known proline hydroxylation targets including collagen, actin and FKBP10 proteins, and many previously unreported proline hydroxylation substrates. Our pathway enrichment analysis results showed that proline hydroxylation substrates are highly enriched in the biological processes including multi-organism processes, macromolecular assembly, RNA splicing, and regulation of response to stress.

In this study, we identified and validated Bromodomain-containing protein 4 (Brd4) as a novel proline hydroxylation substrate in leukemia cells. Brd4 transcription activity is crucial in cancer and metabolic diseases. Understanding the mechanisms that regulate Brd4 transcriptional activity has broad impact in characterizing Brd4-mediated transcriptional networks in cellular processes. PHD inhibition led to significantly decreased prolyl hydroxylation abundance on Brd4 relative to its unmodified peptide (Hyp stoichiometry from 59% to 24% upon DMon treatment). Co-immunoprecipitation experiments revealed BRD4 interaction with the prolyl hydroxylase domain enzyme, PHD2. To confirm enzymatic regulation of Brd4 hydroxylation, overexpression of PHD2 led to a significantly increased BRD4 Hyp stoichiometry (2-fold increase). Functional experiments revealed prolyl hydroxylation reduced Brd4-mediated transcriptional activity. Inhibition of prolyl hydroxylase enzymatic activities significantly diminished the transcription of several of known Brd4 transcriptional targets, c-Myc, Ran and Rad21. DMon treatment significantly reduced Brd4 binding to the c-Myc promoter and significantly inhibited cell proliferation in AML leukemia cells.

MONDAY 3:00 – 4:20 PM
INFORMATICS: EMERGING & NEW APPROACHES, *Ballroom 2*

Mon Talk 3:50-4:05 pm: Improved Protein Inference for Multiple Protease MS Data Using a Single Database Search

Rachel Miller[1]; Connor Hoffmann[1]; Gloria Sheynkman[2, 2]; Robert Millikin[1]; Stefan Solntsev[1]; Anthony Cesnik[1]; Michael Shortreed[1]; Lloyd Smith[1, 3]
[1]University Wisconsin-Madison, Madison, Wisconsin; [2]Center for Cancer Systems Biology (CCSB), Boston, MA; [3]Genome Center of Wisconsin, Madison, WI

Peptides that are detected by tandem mass spectrometry (MS/MS) in bottom-up proteomics serve as a proxy for the proteins expressed in a certain cell or tissue type. In recent years, the use of multiple proteases for protein digestion has grown because it greatly improves sequence coverage. Following MS analysis, peptide identification and protein inference for multiple protease MS data is currently performed separately for each protease, and the results of such analyses are manually aggregated; no existing software allows for peptide identifications from multiple proteases to be used simultaneously for protein inference. We have developed a new software tool that performs peptide identification and protein inference on data from multiple proteases in a single search task. This method improves the overall accuracy of protein inference by eliminating a significant number of protein groups that are erroneously identified by the traditional method. Peptide identifications from these false positive protein groups were reassigned to other groups based on additional evidence for those proteins based on data from alternative proteases. This increased accuracy of protein inference may improve downstream targeted quantitative proteomics and reduce wasted effort in pursuit of erroneously identified proteins.

Mon Talk 4:05-4:20 pm: A sampling of perceptions in the field of mass spectrometry data processing software

Rob Smith
University of Montana, Missoula, MT

Mass spectrometry is an important tool used by many scientists throughout the world. Nonetheless, feedback on the strengths and limitations of current software is often restricted to anecdote rather than formal inquiry. Over the course of interviews with 100 mass spectrometry professionals, surprising patterns coalesced on several software-based topics: perception of the frontier, perception of software quality, and differences between commercial and non-profit environments. Scientists' anonymized responses are presented and summarized into their suggestions for improving the state of the art.

MONDAY 3:00 – 4:20 PM
PROTEOMICS OF CANCER & DISEASE, *Ballroom 1*

Mon Talk 3:00-3:25 pm: Proteomic Analysis of Stalled Replication Forks

Anja Bielinsky[1]; Ya-Chu Chang[1]; Rebecca Rivard[2]; Yee Mon Thu[1]; Susan Kaye Van Riper[1]; LeeAnn Higgins[2]; Todd Markowski[1]; Katarzyna Kulej[2]; Jack Hedberg[1]; Luke Erber[1]; Yue Chen[1]; Eric Brown[2]
[1]University of Minnesota, Minneapolis, MN; [2]University of Pennsylvania, Philadelphia, PA

Ataxia telangiectasia and Rad3-related protein kinase (ATR) is the central player in response to replication stress. Replication stress is caused by conditions that challenge the progression of replication forks and lead to fork stalling. ATR deficiencies in humans are associated with Seckel syndrome, a disease that is characterized by growth retardation and severe microcephaly. In mouse models, mutations in *ATR* result in premature aging phenotypes that have been linked to the replicative exhaustion of stem cells. To better understand the underlying mechanisms of these observations, we sought to characterize ATR deficiency in the context of replication fork stalling. The isolation of proteins on nascent DNA (iPOND) combined with quantitative proteomics allowed us to study the proteomes associated with active and stalled replication forks. Following iPOND, we applied a novel algorithm (RIPPER), coupled with directed tandem mass spectrometry (DRIPPER for *directed* RIPPER) to establish a robust and powerful tool to reveal molecular consequences of ATR deficiency under conditions of replication stress.

Upon challenging cells with the replication inhibitor aphidicolin, stalled forks remained initially stable but relocated to nuclear pores, a known site of DNA repair. After prolonged fork stalling, core replisome components dissociated from chromatin, indicative of fork collapse. Interestingly, this dissociation was dependent on p97 segregase, suggesting that these factors are removed via ubiquitin-dependent proteasomal degradation. In ATR-deficient cells, association of repair factors with stalled forks was delayed, and forks were unable to relocate to the nuclear pore in a timely manner. In summary, these results show that stalled forks are localized to nuclear pores before the replication machinery gets disassembled to allow for the repair of damaged DNA and that ATR is critical for this process. Supported by NIH R01GM074917 and CA189743.

Mon Talk 3:25-3:50 pm: Hunting Circulating Mediators of Systemic Vascular Dysfunction after Carbon Nanotube Exposure

Andrew Ottens
Virginia Commonwealth University, Richmond, VA

Engineered carbon nanomaterials are growing in diversity and popularity for manufacturing strong, lightweight components. Yet, health risk assessments on these materials have only recently begun. Concern over their small size and ease with which they disperse and become trapped within the lung raises questions over their pulmonary as well as extrapulmonary health effects. Recent studies have demonstrated functional consequences outside the lung, and in particular on the vasculature. Blood from exposed animals can cause inflammation and dysfunction in naïve cells and tissues, yet the carbon nanotube material itself is restricted principally to the lung. Studies are ongoing to assess factors introduced into circulation that contribute to systemic health consequences, such as disruption of the blood-brain barrier and induced neuroinflammation. Male C57BL/6 mice were exposed to two doses of multi-walled carbon nanotubes (Matsui-7, 49

nm mean diameter) or vehicle (n=6/group). Samples were size fractionated and assessed using label-free data independent analysis. Mass spectrometry revealed over 1,700 circulating factors significantly altered by nanotube exposure that correlated with levels present within bronchial lavage fluid. Lung tissue showed significant 2-fold or greater increases in 12 matrix proteases supporting a pulmonary origin of peptide proteolytic products released into circulation. Peptide analysis identified products from known matrix protease substrates and secretory proteins, which offered functional relevance to immuno-inflammatory responses, extracellular matrix remodeling, and receptor interactions. The tested fraction was further evaluated and found to induce inflammatory marker release in naïve endothelial cells as well as vasodilatory dysfunction in naïve blood vessels. Of particular interest was a 58-mer thrombospondin fragment that contains antiangiogenic CD36 binding characteristics as needed to induce the observed vascular dysfunction. Results from these studies underlie an indirect mechanism by which pulmonary insults, such as to nanomaterials, can translate into systemic health consequences.

Mon Talk 3:50-4:05 pm: Polycomb loss enhances oncogenesis but leads to therapeutic vulnerabilities in malignant peripheral nerve sheath tumors

John Wojcik[1, 2]; Dylan Marchione[1, 2]; Simone Sidoli[1, 2]; Ben Garcia[1, 2]
[1]University of Pennsylvania, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA

Malignant peripheral nerve sheath tumors (MPNST) are aggressive sarcomas in which loss of function mutations in the polycomb repressive complex 2 (PRC2) promote tumor progression. To better understand how PRC2 loss contributes to pathogenesis, we conducted parallel proteomic and epigenomic analysis of human MPNSTs with and without PRC2 loss ($MPNST_{LOSS}$ vs. $MPNST_{RET}$).

$MPNST_{LOSS}$ showed decreased H3K27me3 and increased of H3K27 acetylation. This was accompanied by hyperacetylation of H4, a marker of open chromatin, and increased H3K36me2 and DNA methylation. At the level of the proteome, $MPNST_{LOSS}$ had an increased abundance of chromatin remodelers and markers of cell growth and division and decreased interferon signaling and antigen presentation.

To assess whether PRC2 function was directly linked to the proteome changes seen in human tumors, we restored PRC2 function in $MPNST_{LOSS}$ cell lines and measured the proteome response. PRC2 reconstitution downregulated pathways that were upregulated in $MPNST_{LOSS}$ and increased interferon pathway expression and MHC presentation. The same effects were observed in $MPNST_{LOSS}$ cell lines with knockdown of NSD2, the methyltransferase responsible for H3K36me2, suggesting functional antagonism between H3K36me2 and H3K27me3.

NSD2 knockdown also decreased proliferation and DNA methylation and activated transcription of endogenous retroviral elements (ERV), thereby linking H3K36me2 loss with interferon activation, which occurs as a consequence of ERV expression. $MPNST_{LOSS}$ were found to be highly sensitive to both DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi), both of which similarly activate interferons through induction of ERVs.

Together, these results suggest that PRC2 loss promotes global increases in open chromatin that enhance oncogenic pathway expression but promote genomic instability, and render MPNST heavily reliant on DNA methylation to prevent spurious transcription initiation, including of ERV. Consequently, MPNST are highly sensitive to therapeutics promoting further destabilization through increased acetylation (HDACi) or decreased DNA methylation (DNMTi).

Mon Talk 4:05-4:20 pm: A proteomics approach to understand the role of autophagy in colorectal cancer and enhance chemosensitivity

Emily Cannon; Monique Speirs; John Price
Brigham Young University, Provo, Utah

Colorectal cancer (CRC) is the third leading cause of cancer deaths, and CRC recurrence and chemoresistance limit the 5-year survival rate to 8%. Understanding how metabolic adaptations promote the transition from normal to malignant colon cells is necessary to improve treatments, and identify chemosensitizing drug targets. Autophagy is a conserved protein degradation pathway by which cytosolic components are engulfed, degraded by lysosomes, and recycled. Cancer cells respond to microenvironmental and treatment-induced stress by increasing autophagy-driven protein turnover. This increase is linked to stress tolerance and chemoresistance by unknown mechanisms. Identifying whether autophagy is selective for specific proteins and/or organelles is crucial to understand how autophagy provides a metabolic advantage in CRC. We are using kinetic proteomics techniques to measure autophagy and its effects on the turnover of specific protein types in human colon tumor (HCT116) cells. Our data suggest that autophagy is selective in CRC and plays key roles in stress tolerance, growth, and resistance to conventional CRC chemotherapeutics. Distinguishing between definitive autophagy substrates at the proteome level will help us identify specific anti-autophagy targets that may be used to sensitize resistant CRC cells to chemotherapy.

MONDAY 4:30 – 5:50 PM
QUANTITATIVE PROTEOMICS I, *Ballroom 2*

Mon Talk 4:30-4:55 pm: Drug Target Identification by Label-Free Differential Mass Spectrometry

Nathan Yates
University of Pittsburgh, Pittsburgh, PA

Few analytical techniques offer as many possibilities for the identification of novel drug targets as proteomics. Here we describe the development of label-free differential mass spectrometry (<g class="gr_ gr_10 gr-alert gr_spell gr_inline_cards gr_run_anim ContextualSpelling ins-del multiReplace" id="10" data-gr-id="10">dMS</g>) as a sensitive and selective method for the unbiased identification of proteins that bind to small molecule drugs. This lecture will highlight the application of our <g class="gr_ gr_11 gr-alert gr_spell gr_inline_cards gr_run_anim ContextualSpelling ins-del multiReplace" id="11" data-gr-id="11">dMS</g> target identification platform to different classes of approved drugs (e.g. NSAIDs, metformin, and carbonic anhydrase inhibitors) as well as novel cancer therapeutics (e.g. <g class="gr_ gr_12 gr-alert gr_spell gr_inline_cards gr_run_anim ContextualSpelling ins-del multiReplace" id="12" data-gr-id="12">mdivi</g>-1). Using the approach, we have identified known and novel targets that have been confirmed by quantitative western blot and functional assays. We have found the approach to be quite efficient and versatile, allowing for up to five compounds to be screened in less than a week on a single mass spectrometer. A strength of the label-free <g class="gr_ gr_13 gr-alert gr_spell gr_inline_cards gr_run_anim ContextualSpelling ins-del multiReplace" id="13" data-gr-id="13">dMS</g> approach is the precise quantification of all features that are detected in full-scan high-resolution mass spectra, not just peptides that have been identified via MS/MS experiments. Results are ranked by statistical significance and statistical power can be easily adjusted by increasing sample size. Overall, our results support the utility of proteomics for target identification and demonstrate a straightforward method that can be readily carried out as part of an efficient phenotypic screening drug development strategy.

Mon Talk 5:20-5:35 pm: FlashLFQ: Ultrafast Label-Free Quantification of Peptides in Proteomics

Robert J. Millikin; Stefan K. Solntsev; Michael R. Shortreed; Lloyd M. Smith
University of Wisconsin, Madison, WI

The rapid and accurate quantification of peptides is a critical element of modern proteomics that has become more challenging as proteomic data sets increase in size and complexity. FlashLFQ is a computer program for high-speed label-free quantification of peptides following a search of bottom-up mass spectrometry data. It is approximately an order of magnitude faster than established label-free quantification methods, making it practical to base quantification upon all charge states for a given peptide rather than solely upon the charge state that was selected for MS2 fragmentation. This increases the number of quantified peptides, improves replicate-to-replicate reproducibility, and increases quantitative accuracy. FlashLFQ is integrated into the MetaMorpheus search software GUI, allowing it to work together with the global post-translational modification discovery (G-PTM-D) engine to accurately quantify modified peptides. It is also available as a NuGet package, facilitating its integration into other software, and as a

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