New Technology for the Large-scale Proteomic Comparison of Human Embryonic Stem Cells, Induced Pluripotent Stem Cells, and Somatic Cells

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Induced pluripotent stem cells (iPS) represent a breakthrough in stem cell research and pose remarkable therapeutic potential. They circumvent ethical issues surrounding the use of human embryonic stem cells (ES) and could eliminate immune rejection in transplantation therapies. In this work we investigate similarities and differences between human ES and iPS cells that may affect the use of iPS cells for research and therapeutic purposes. To perform these experiments we use novel mass spectrometry-based analyses in combination with isobaric tags for absolute and relative quantitation (iTRAQ). To date, we have identified 77,959 unique peptides and 6,534 unique proteins. The fold-differences observed between pluripotent lines and the somatic line showed a high correlation (R = 0.91) demonstrating remarkable similarity between ES and iPS cells. The set of proteins that was expressed at higher levels in pluripotent cells was mostly nuclear in nature and functionally enriched in the processes of transcriptional regulation and chromatin modification. Among these were numerous transcription factors and other proteins important to the maintenance of pluripotency including SOX2, OCT4, DPPA4, and LIN28. In agreement with current literature, comparison of protein expression changes to changes in mRNA expression revealed only a weak correlation (R = 0.68) highlighting the need for quantitative proteomic analysis.

Electron Capture Dissociation in Radio-Frequency-Free Cell

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A radio-frequency-free (RFF), analyzer-independent cell has been devised for electron-capture dissociation (ECD) of ions. The device is based on interleaving a series of electrostatic lenses with the periodic structure of magnetostatic lenses commonly found in a traveling wave tube. A five-magnet version of the RFF electromagnetostatic ECD cell was installed in a Finnigan TSQ700 ESI triple quadrupole (QqQ) spectrometer, and its performance was evaluated by recording product-ion spectra of various peptides. These spectra were readily obtained without recourse to a buffering gas or synchronizing electron injection with a specific phase of an RF field. The mass spectra produced with the modified instrument appear in all respects (other than resolution and mass accuracy, which were limited by the mass spectrometer used) to be at least as good for purposes of peptide identification as those recorded with Fourier transform ion cyclotron resonance (FT ICR) instruments; however, the effort and time to produce the mass spectra were much less than required to produce their FT ICR counterparts. A two-magnet version of the electromagnetostatic ECD cell was installed in the same mass spectrometer and used to simultaneously obtain combined ECD/CID product-ion mass spectra that exhibit a-, b-, and c-type ion signals. Details of the cell design, construction, and operation will be presented and discussed.

Decoding the Histone Code by Quantitative Proteomics

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Epigenetic refers to stable heritable changes in gene expression that are not due to changes in DNA sequence, such as DNA methylation, RNA interference and histone modifications. Histones are small basic proteins that function to package genomic DNA into repeating nucleosomal units (containing ~146 bp of DNA wrapped around two copies of each of histones H3, H4, H2A and H2B) forming the chromatin fiber and hence our chromosomes. In general, the packaging of DNA into chromatin is recognized to be a major mechanism by which the access of genomic DNA is restricted. A wide number of studies show that several covalent histone modifications such as methylation, acetylation, phosphorylation and ubiquitination located in the N-terminal tails correlate with both the regulation of chromatin structure during active gene expression, Oregon, USA heterochromatin formation during gene silencing. Here we are developing novel proteomic strategies to discover differentially expressed histone modifications, identify concurrent combinatorial histone modifications (Histone Codes), and characterize histone codes that are important various processes.
MS.4
Data Processing Algorithms for Analysis of High Resolution MSMS Spectra of Peptides with Complex Post-Translational Modifications
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Data analysis of fragmentation spectra of heavily modified large peptides and small proteins is a challenging task. Efficient fragmentation method, such as electron capture dissociation (ECD) or electron transfer dissociation (ETD), allows for more uniform cleavage along all peptide backbones and for retention of many labile modification groups. However, MSMS spectra from large peptides may contain hundreds perhaps thousands of often overlapping isotopic clusters. We have developed a strategy to directly match the theoretical isotopic distributions to the experimental data. The so-called Fragment Assignment by Visual Assistance (FAVA) program detects more fragment ions than by use of deisotopic algorithms.

Heavily modified peptides such as histones, add additional difficulty for the data analysis. Many MSMS spectra come from a mixture of peptides with the same molecular weights but different PTM arrangement. In order to distinguish those PTM “variants” and to estimate their relative stoichiometry, we have extended our FAVA algorithms to examine possible PTM combinations.

We have tested our processing programs with large peptides from Histone samples. Those peptides contain up to 12 lysine methylations. Acetylations and trimethylations can be differentiated by mass accuracy and identification and stoichiometry determination accuracies can be estimated from numbers of unique ions identified and from their intensities.

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MS.5
Use of Electron Transfer Dissociation to Analyze Combinations of Histone Post-Translational Modifications on an LTQ-Orbitrap
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Post-translational modifications of histones are used to regulate DNA-chromatin interactions and ultimately gene expression. In many cases, the various histone PTMs do not regulate function individually. Instead, combinations of modifications are believed to act together to create a ‘histone code’. Elucidating these combinations through analyses of their proteolytic digests since is often not possible as much of this combinatorial information is lost following protein digestion. It is essential to be able to detect and assign the sites of these modifications in combination on particular protein isoforms to assess their significance.

Accordingly, we have developed approaches for both direct infusion and online reverse phase liquid chromatography separation for intact histone and large histone peptides (AspN or GluC digests) on an LTQ Orbitrap with electron transfer dissociation (ETD) fragmentation.

We have applied these methods for the analysis of post-translational modifications of histones in methyltransferase knockout mouse embryonic stem cells compared with wildtype cells. Combined with stable isotope labeling of amino acids in cell culture (SILAC), changes in stoichiometries of modification site occupancies are revealed by quantitative mass spectrometry. Through these techniques, we show that knock out of a specific methyltransferase leads to a variety of histone modification changes.

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Electron Capture Dissociation for Structural Studies of Integral Membrane Proteins and Their Modifications

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Full structural analysis of the c-subunit of the Fo domain of ATP synthase demonstrated that the integral membrane proteins that constitute around one third of the proteome are amenable to top-down high-resolution mass spectrometry. Effective electron capture dissociation (ETD) was achieved through thermal excitation of ions using an infra-red laser for activated-ion, or aECD, implying that it was necessary to break alpha-helix hydrogen bonds prior to electron capture. Collissionally activated dissociation (CAD) was also effective for top-down analysis of the c-subunit but aECD clearly improved sequence coverage, allowing substantial sequence coverage within the two alpha-helical transmembrane domains. The need to use thermal excitation for effective ECD of this 8 kD protein suggested that integral membrane proteins may have a lower threshold for this requirement than soluble proteins. Subsequent studies of a variety of integral membrane proteins have not been consistent enough to answer this question. The most consistent feature of these experiments has been the frequent observation that while CAD yields useful product ion spectra ECD most often yields familiar charge reduction series even when IR laser is used to activate the ions. The origin of this problem seems to be the fact that integral membrane proteins typically carry fewer charges than soluble proteins after electrospray ionization giving them higher m/z. Strategies to achieve higher charging will be discussed.

O-GlcNAcylation: The Post-Translational Modification that Best Highlights the Value of ETD

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O-GlcNAcylation is a widespread regulatory modification of nuclear and cytoplasmic proteins, analogous to phosphorylation. For a modification that was first discovered 25 years ago, surprisingly little is known about how it regulates protein functions, despite its clear role in problems such as diabetes and Alzheimer disease. Slow progress in characterizing this modification has largely been due to a lack of effective methods for detecting and locating O-GlcNAcylation sites. This is in a large part because the O-glycosidic link that attaches the single N-acetylglucosamine (GlcNAc) moiety to serines and threonines is highly labile under collision induced dissociation in a mass spectrometer (much more so than phosphorylation), so O-GlcNAc site assignment using this mass spectrometric approach has proven largely unsuccessful.

Electron transfer dissociation (ETD) is a recently developed radical-based fragmentation technique that fragments components at locations not defined by bond strength, so is able to maintain labile modifications on fragment ions. Hence, the modification is not cleaved during ETD mass spectrometric analysis, allowing O-GlcNAcylation site assignment.

In this talk I will present how ETD availability, along with strategies for enriching for modified peptides, is starting to transform the characterization of this modification.

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The Use of ECD for Proteomics-wide Identification and Quantification of iso-Asp Residues

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Protein deamidation is one of the major post-translational modifications (PTM) that lead to protein inactivation in vivo. It has been shown to relate with neurodegenerative diseases (e.g. Alzheimer’s disease) and the degradation of commercial protein products. Asparaginyl residue is a major source of deamidation in biological samples, the minor one being the glutaminyl residue. Asparagine deamidation is a non-enzymatic PTM that occurs spontaneously under physiological conditions, which results in a mixture of aspartyl (Asp) and isoaspartyl (isoAsp) residues. Asp isomerization also contributes to the increase in the isoAsp pool, albeit at a lower rate.

Electron capture dissociation (ECD) combined with Fourier transform mass spectrometry (FT MS) are able to distinguish the isoaspartyl peptides by unique ECD fragments of c\textsubscript{n} + 58.0054 (C\textsubscript{16}H\textsubscript{2}O\textsubscript{2}) and z\textsubscript{n} – 56.9976 (C\textsubscript{8}H\textsubscript{8}O\textsubscript{2}), where n is the position of the aspartyl residue and f is the peptide length.

In the present study, we tested the specificity of isoAsp detection using the accurate masses of these specific fragments. Totally, 466 isoAsp peptide candidates were identified from 32 whole and partial human proteome samples. Then additional criteria, like adjacent c/z fragments, specific losses from the reduced species, and the shape of the chromatographic peak, were applied to increase the specificity of the method. Upon detailed inspection, 219 isoAsp peptide candidates have been supported by at least one criterion other than the mass of the specific ECD fragments. Most stringent filtering of these candidates yielded several cases where the presence of isoAsp was beyond doubt. Among the identified proteins with isoAsp, actin, heat shock cognate 71 kDa protein and pyruvate kinase have previously been identified as substrates for L-isoaspartyl methyltransferase (PIMT), an important repair enzyme converting in vivo isoaspartyl to aspartyl. Quantification of relative isomerization degree was performed by the label-free approach using in-house developed software. This is the first attempt to analyze the human isoaspartome in a high-throughput manner. The developed workflow allows for further enhancement of the detection rate of isoaspartyl residues in biological samples.