Detection of Age-Related PTMs as *m/z* Pair Tags in LC-MS Measurement of Mesenchymal Stem Cell Lysates

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Overview

Purpose: To discover age-related post-translational modifications (PTMs) in proteins from mesenchymal stem cell extracts.

Methods: Samples were analyze using the BRIMS Two-Pass Workflow [1] including high-resolution MLC-MS/MS analysis on a hybrid ion trap – Orbitrap mass spectrometer.

Results: The two-pass workflow combined with SIEVE PerfectPair algorithm complements standard data-dependent fragmentation PTM identification. A differential pattern of PTMs is described in young versus old mesenchymal stem cell lysates.

Introduction

Post-translational modifications are important in protein signaling cascades and mechanisms. Detection of PTMs in LC-MS experiments is often achieved by peptide sequencing enabled by ion fragmentation. In this case, detection of PTMs is limited by the ability to interpret fragmentation spectra. In addition, fragmentation spectral interpretation and sequencing using tools such as SEQUEST® or Mascot™ is time consuming and requires *a priori* knowledge of the PTMs for their detection.

A better understanding of posttranslational modifications can be important for understanding cellular processes, including aging. We describe a method to detect PTM candidates without fragmentation sequencing by identifying mass spec full scan peak pairs that are consistent in *m/z* and retention time with the modified and unmodified form of the ion.

Methods

Samples

Mesenchymal stem cells are derived from human adipose tissue. Two sample lysates are prepared from cell cultures at different ages (Young and Old). The samples (8 µL, 200 ng) were injected into a Thermo Scientific EASY-nLC system configured with a 5 cm x 100 µm trap packed with 15-20 µm PS-DVB 300 A media, and a 25 cm x 100 µm ID resolving column packed with 200 A, C18 AQ media. Samples were loaded at 5 µL/min for 9 min, and a gradient from 0-60% B at 375 nL/min was run over 70 min, for a total run time of 115 min (including regeneration, and sample loading).

A Thermo Scientific LTQ Orbitrap Velos hybrid mass spectrometer equipped with electron-transfer dissociation was run in a top-20 data-dependent mode, with a full-scan run in the Orbitrap™ mass analyzer at 60K resolution (1e6 target), with up to 10 MS2 events. Monoisotopic precursor selection, along with rejection of unknown, +1, and >4+ ions was used in precursor ion selection. Standard siloxane and phthalate ions were used as lock masses (*m/z* 371, 391, 445). Data were processed using Thermo Scientific SIEVE software and Thermo Scientific Proteome Discoverer software.

Results

Data were acquired using the BRIMS Two-Pass Workflow (Figure 2, above right) where first-pass acquisition focuses on full scans and second pass is based on a targeted fragmentation focused run where the targets are the result of a SIEVE™ [2] analysis.

BRIMS Two-Pass Acquisition

We found that the chromatography and instrument methods for optimal full-scan quantitative measurements conflicted with methods for optimal fragmentation scans. Therefore, we exploited the mass spectrometer's mass accuracy and broad dynamic range by taking two distinct passes of data measurement. The first pass focused upon acquiring uncompromised and optimized full-scan (MS) data for highly reproducible quantification. This first full-scan quantitative pass was used to generate an inclusion list of potentially interesting features. The inclusion list was then used for targeted fragmentation scan acquisition during a second pass of a subset of the data samples.

Pass 1

Five technical replicate injections each of Young and Old mesenchymal stem cell lysates were processed in a full-scan optimized configuration. Each replicate set was analyzed using the SIEVE v1.3 PerfectPair processor – a frame annotation algorithm that determines pairs of frames (features) that are related in mass and retention time differences (Figure 1).

FIGURE 1. *m/z* **pair tags (PerfectPairs) are two distinct features (frames) whose** *m/z* **and retention time differences are consistent with a pre-defined value, such as a PTM. PerfectPairs can also be used in tagging isotopically labeled pairs and adducts.**

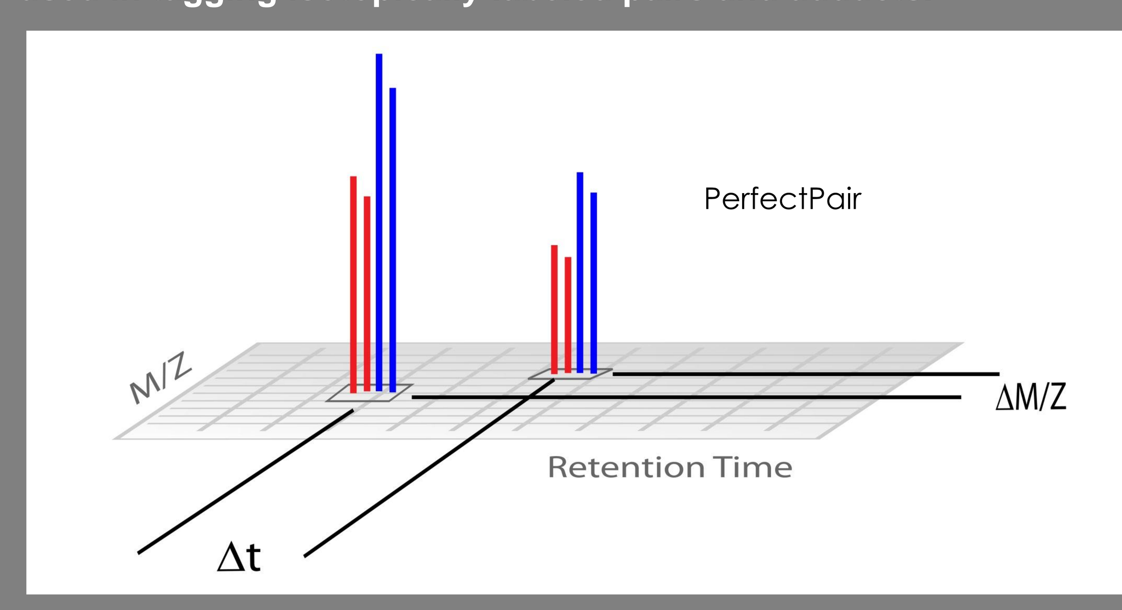


FIGURE 2. BRIMS Two-Pass Workflow : A) High-quality full-scan acquisition with five technical replicates on the EASY-nLC and LTQ Orbitrap Velos MS including a top-20 data-dependent method, B) Label-free processing with SIEVE software including PerfectPair analysis to produce an inclusion list, C) Targeted acquisition with high quality fragmentation scans, D) ID analysis with SEQUEST, Proteome Discoverer , and SIEVE.

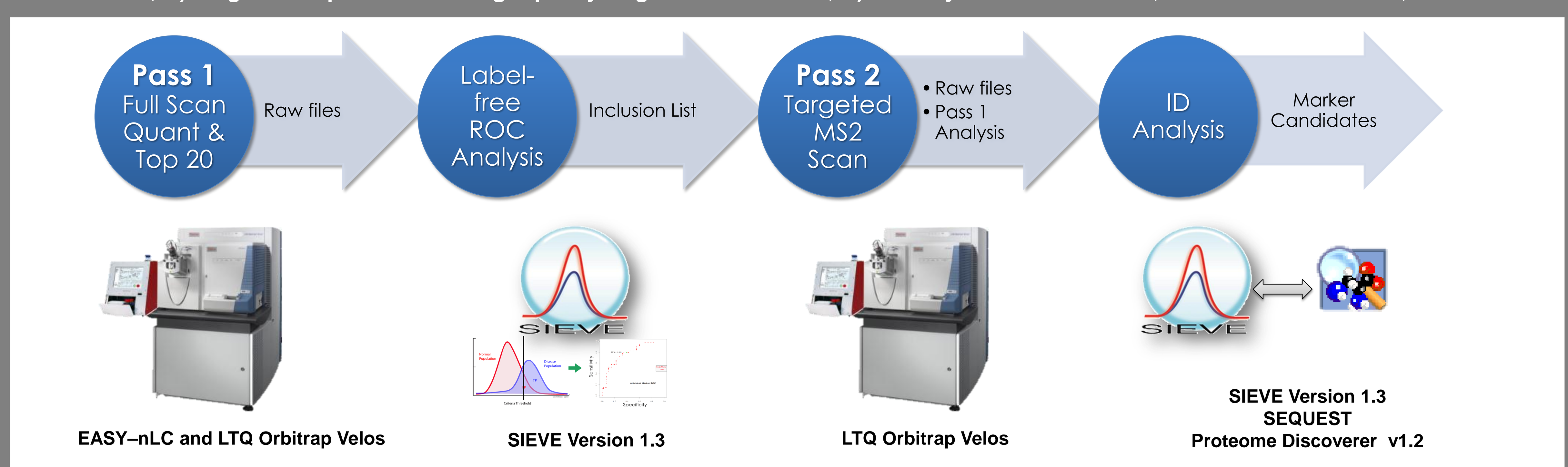
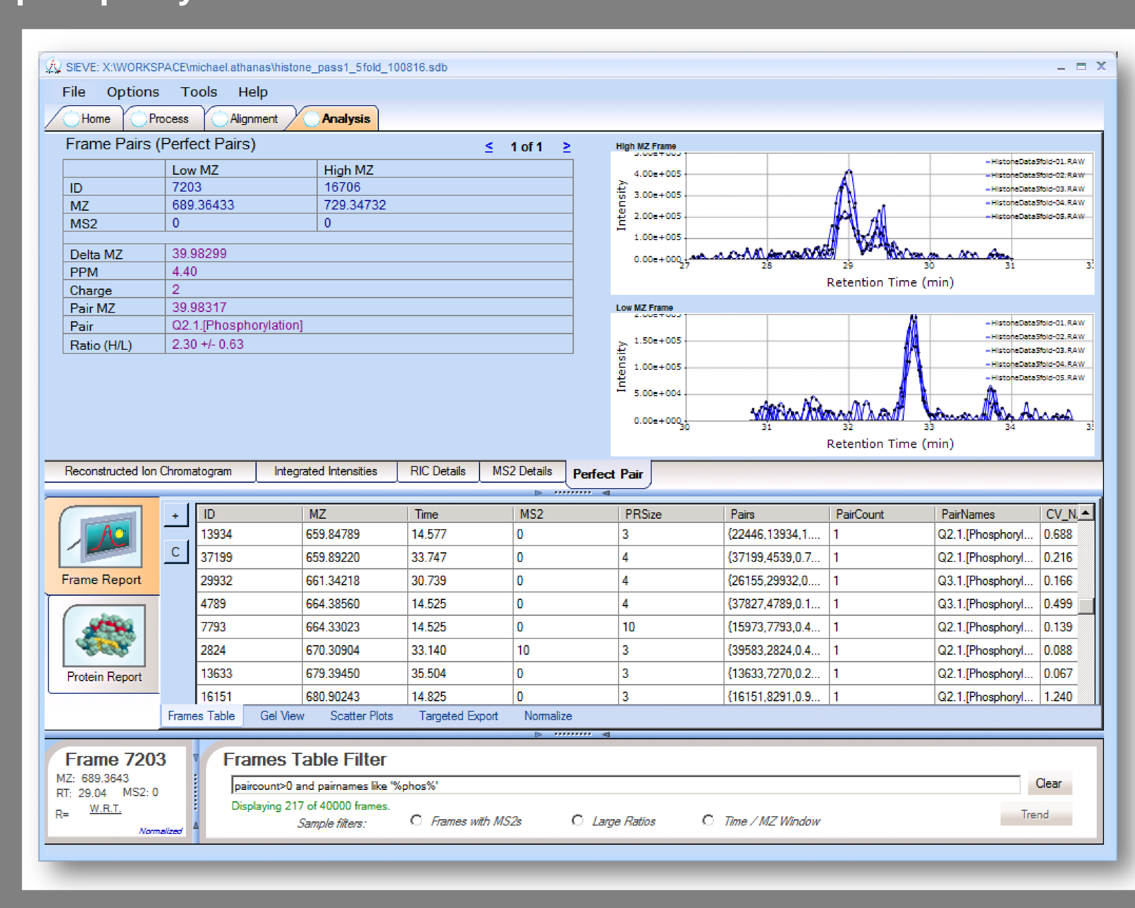


FIGURE 3. Screen capture from SIEVE v1.3 label-free analysis platform illustrating the PerfectPair viewer. Two frames with differences in *m/z* **and retention time that are consistent with phosphorylation are shown .**

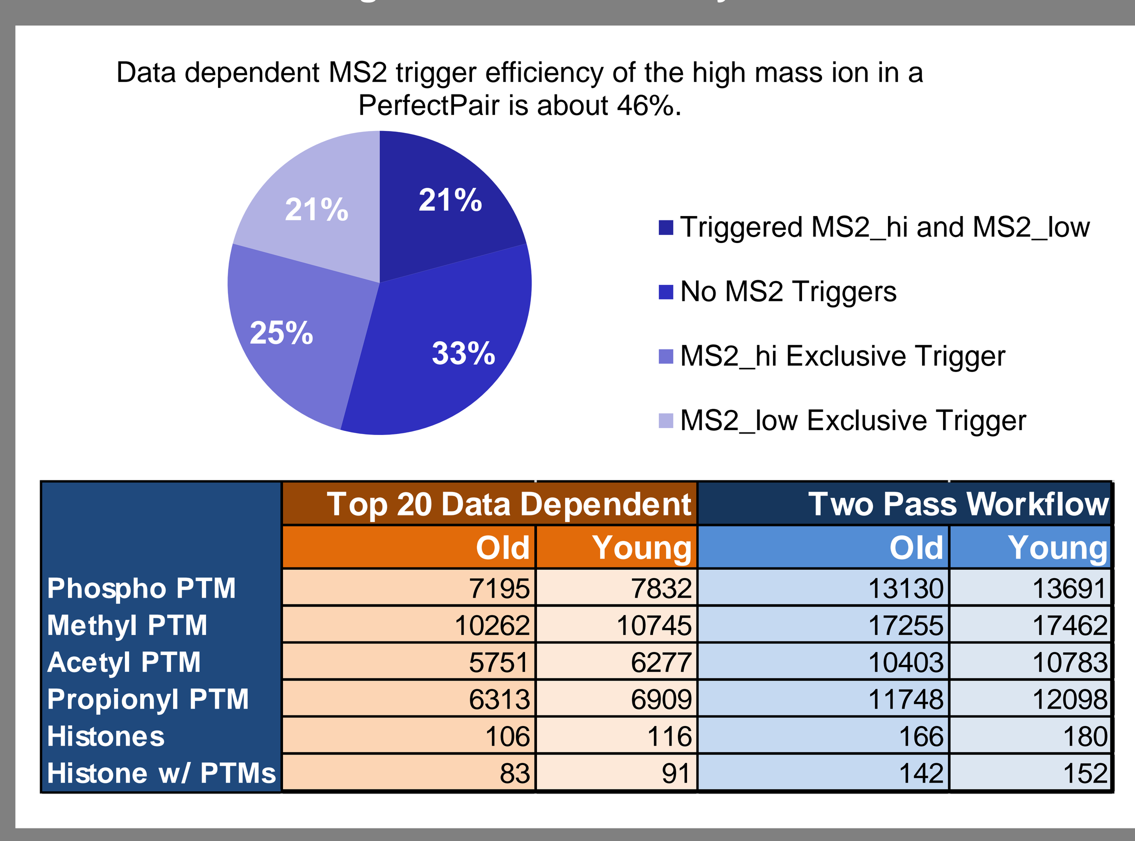


Peak candidate pairs were collected with the following characteristics:

A. Mass difference of the pairs is consistent with methylation, acetylation, phosphorylation, lysine propionylation, or lysine propionylmethylation to within 7 ppm
B. Peak elution times must be within 7 minutes
C. Peaks are limited to charge 2 or 3
D. Only consider cases where there are one or two modifications per peak

The SIEVE PerfectPair processor discovered 1174 and 1193 pairs for the Old and Young samples (Figures 3 and 4). An inclusion list was generated from these pairs for two separate pass 2 analyses. Data-dependent fragmentation scans were also acquired in the Pass 1 acquisition using a top-20 method.

FIGURE 4. TOP. Data-dependent fragmentation trigger rates of phosphorylated peptides are compared to rates expected from a full-scan analysis using the PerfectPair algorithm. MS2_hi and MS2_lo refer to the triggering of the high mass / modified ion and the unmodified ion, respectively. BOTTOM. Identified PTMs compared with standard top-20 data dependent and two-pass workflows illustrating increased sensitivity.



	Top 20 Data Dependent		Two Pass Workflow	
	Old	Young	Old	Young
Phospho PTM	7195	7832	13130	13691
Methyl PTM	10262	10745	17255	17462
Acetyl PTM	5751	6277	10403	10783
Propionyl PTM	6313	6909	11748	12098
Histones	106	116	166	180
Histone w/ PTMs	83	91	142	152

FIGURE 5. Subset of identified peptides with methylation and acetylation in Young and Old mesenchymal stem cell lysates.

Unique Peptide Sequence	Description	Modification	Old	Young
DFLAGGVAAAVSk	ADP/ATP translocase 1 [Homo sapiens]	C-Term(Methyl)	X	
RESISELISLr	cholesterol 7-alpha-monooxygenase [Homo sapiens]	C-Term(Methyl)	X	
LFIGGLSFETTDdSLR	heterogeneous nuclear ribonucleoprotein A3 [Homo sapiens]	D13(Methyl)	X	
IGFGSFVdK	integrin beta-2 precursor [Homo sapiens]	D8(Methyl)	X	X
KEEeLQGALAR	myosin-10 [Homo sapiens]	E4(Methyl)		X
ISMPDVDLHVk	neuroblast differentiation-associated protein AHNAK isoform 1 [Homo	C-Term(Methyl)	X	
aAAAAATAAAAASIR	sec1 family domain-containing protein 1 isoform a [Homo sapiens]	N-Term(Acetyl)	X	
VNIIPVIAk	septin-10 isoform 2 [Homo sapiens]	C-Term(Methyl)	X	X
TLMALGSVAVTk	transgelin-3 [Homo sapiens]	C-Term(Methyl)	X	
AVFVDLEPTVVDEVr	tubulin alpha-3C/D chain [Homo sapiens]	C-Term(Methyl)	X	X
LAGTQPLEVLEAVQR	ubiquitin-like modifier-activating enzyme 1 [Homo sapiens]		X	
STdLILR	vomeronasal type-1 receptor 2 [Homo sapiens]	D3(Methyl)	X	X

Pass 2

Higher concentration samples were injected with emphasis on quality MS2 spectra. Only one sample from each cell line was analyzed with each inclusion list. Resulting pass 2 spectra are matched to SIEVE pass 1 frames. The MS target list acquisition efficiency is about 99%; that is, only 12 of the 1174 inclusion list targets failed to trigger a fragmentation scan.

PTM / peptide identification

The criteria used to assess the performance of the BRIMS Two Pass approach were based on the total number of high-quality distinct peptide identifications found. The SEQUEST algorithm within Proteome Discoverer™ software was configured for peptide identification with the appropriate dynamic modifications. A peptide probability score of 10 or higher distinguished quality ID assignments. IDs were acquired from the top-20 fragmentation data in Pass 1 and the targeted fragmentation scans of Pass 2. The two sets of IDs were compared.

Conclusion

Peptides were identified in both the top-20 data dependent pass 1 data set as well as the targeted pass 2 data set. Comparing the two sets of high-quality identifications can be used to assess the performance of the PerfectPair and BRIMS Two-Pass Workflow. In this case, combining the ID results from the pass 2 and pass 1 results in significantly more high-quality PTM IDs (Figure 5).

- The BRIMS Two-Pass Workflow combined with the SIEVE v1.3 PerfectPair algorithm can uncover significantly more candidate peptides than a data-dependent workflow alone.
- The SIEVE PerfectPair algorithm complements the fragmentation identification method for finding PTMs; however, PerfectPair alone is not helpful when the unmodified form is absent or undetectable.
- A differential pattern of PTMs is described in young *versus* old mesenchymal stem cell lysates.

References

1. Michael Athanas, David Sarracino, Taha Rezai, Amol Prakash, Jennifer Sutton, Bryan Krastins, Mingming Ning, Mary F Lopez *"A Two-pass Informatics-driven Label-free Workflow For Discovery Of Neurovascular Mediators In PFO Related Stroke"*, ASMS 2010 Poster
2. V Lunyak, A Prakash, M Athanas, B Krastins, T Rezai1, D Sarracino1, M F Lopez; *Development of a quantitative SRM assay for Histone H2AX Ser139 phosphorylation, a key regulator of the DNA damage response (DDR).* ASMS 2010 Poster

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