Overview

Purpose: Develop a multiplexed, quantitative SRM assay for histones and histone modification proteins for profiling protein abundance in self-renewing and senescent mesenchymal stem cells.

Methods: A multiplexed SRM assay for gamma Histone H2Ax and nine histone modification proteins was developed in one day using heavy-labeled synthetic peptides (gamma Histone H2Ax) and recombinant proteins (nine histone modification proteins). Mesenchymal stem cell lysates from self-renewing and senescent cultures were digested with trypsin and analyzed on a triple-quadrupole mass spectrometer. Peptide abundance ratio patterns were analyzed with software that facilitates quantitative verification of putative biomarkers and general quantitative proteomics, as well as with pathway analysis software.

Results: Dramatic changes in protein abundance were observed between the groups.

Introduction

Histone post-translational modifications (PTMs) are a central theme in the regulation of gene expression. A rapidly growing list of modifications confirms that they play fundamental roles in chromatin remodeling processes. Chromatin structure and function plays a key role in DNA damage repair. The response to double-strand breaks (DSB) induced by radiation or DNA-damaging agents typically results in DNA-damage response (DDR) and accumulation of the proteins involved in DNA repair in subnuclear foci. The rapid phosphorylation of Ser139 in the C-terminus of the H2Ax sequence functions in the recruitment of DDR proteins after DNA damage. These processes are also thought to play a role in stem cell development and senescence. To date, most studies in this area have been carried out by genomic analysis, immunostaining, or top-down LC-MS/MS analysis, and as such are not fully quantitative.

Over the years, mass spectrometry (MS) has emerged as a powerful analytical technique for the identification of unknown compounds, determination of molecular weights, elucidation of post-translational modifications, and quantification of a wide variety of analytes. Although MS has been applied to proteomics research for a long time, it is only recently that it is becoming a powerful tool for the measurement of protein biomarkers. The advantages of SRM-based MS experiments include very high throughput, sensitivity, specificity, and absolute quantification capability, which are generally not achievable with traditional MS approaches. SRM experiments also represent a powerful technique for the measurement and quantification of PTMs and disease-specific biomarkers. MS is the only technique that can derive the specificity required to detect isofoms associated with protein sequence microheterogeneity and many clinically relevant variants. Such arrays could potentially be used for disease diagnosis and prognosis, and they are typically robust and selective, even in complex matrices.

In this study, we developed a multiplexed, quantitative SRM assay for nine histone modification proteins and histone gamma H2Ax. The assay was used to interrogate mesenchymal stem cells derived from human adipose tissue. Lysates were prepared from cells at different ages (self-renewing and senescent) and senescence phases, allowing us to confirm and quantify previously identified PTMs occurring on histone gamma H2Ax, and to demonstrate a dramatic decrease in abundance of histone modification proteins not previously reported in conjunction with somatic stem cell aging.

Methods

Sample Preparation

Human adipose stem cells (hADSCs) were isolated from human subcutaneous white adipose tissue collected during liposuction procedures and herein referred to as “self-renewing hADSCs” using the Thermo Scientific ProteoJET Cytoplasmic and Nuclear Protein Extraction Kit, according to the manufacturer’s protocol. Sample digestion and MS analysis were performed using Ingenuity Pathways Analysis (IPA®) software.

Results

Differential Abundance of Histone and Histone Modification Proteins in Cytoplasmic and Nuclear Fractions from Self-Renewing and Senescent Mesenchymal Stem Cells

Table 1 shows the proteins and peptide sequences used in the multiplexed assay. Surrogate peptides were chosen based upon optimization of parameters in iterative experiments using recombinant proteins or based upon previously obtained discovery data. Table 2 provides the fold change and statistically significant p-values for the quantified proteins. Figure 1 shows the protein abundance ratios of senescent/h self-renewing(SR) in cytoplasmic and nuclear fractions. As is evident in the figure, the abundance ratio profile was dramatically different between the two groups. WHSC1L1, WHSC1 and EP300 had abundance ratios $>1$ in cytoplasmic senescent fractions, whereas SUV420H1, EP300, H2AX phosphorylated on ser 139 (KAT6A)(gamma) (K2057), EZH2 and H2AX phosphorylated on ser 139 (KAT6A)(gamma) (K2057) had increased abundance in nuclear senescent fractions. SUV420H1, MYST2 and H2AX phosphorylated on ser 139 (KAT6A)(gamma) (K2057) were not significantly differentially expressed. The H2AX abundance profile was consistent with the previously published evidence that rapid phosphorylation of Ser139 at the C-terminus of the H2AX sequence functions in the recruitment of DDR proteins after DNA damage. It is hypothesized that increased DNA damage occurs upon senescence.

![FIGURE 1](image1.png)

**FIGURE 1.** Abundance ratios of histones and histone modification proteins in self-renewing (SR) and senescent (S) mesenchymal stem cells.

A. Cytoplasmic fraction, B. nuclear fraction

**TABLE 1.** Proteins and peptide sequences quantified in the multiplexed SRM assay.

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Peptide Sequence</th>
<th>SR/Se (Fold)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histone H2AX</td>
<td>TSNLDK</td>
<td>1.12</td>
<td>0.001</td>
</tr>
<tr>
<td>Histone H2AX</td>
<td>TSNLDK</td>
<td>1.35</td>
<td>0.002</td>
</tr>
<tr>
<td>Histone H2AX</td>
<td>TSNLDK</td>
<td>1.23</td>
<td>0.003</td>
</tr>
<tr>
<td>Histone H2AX</td>
<td>TSNLDK</td>
<td>1.18</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**TABLE 2.** Functional description of the proteins quantified in the SRM assay

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2AX</td>
<td>Repair DSB and radiation response</td>
</tr>
<tr>
<td>MYST2</td>
<td>Histone acetyltransferase, chromatin remodeling</td>
</tr>
<tr>
<td>SUV39H1</td>
<td>Histone methyltransferase, chromatin remodeling</td>
</tr>
</tbody>
</table>

**Conclusion**

The experiments presented in this poster present evidence suggesting differential distribution and abundance of histone H2Ax and histone modification proteins in senescent and self-renewing mesenchymal stem cells. The data demonstrate:

- Development of a multiplexed, quantitative SRM assay for nine histone modification proteins and histone gamma H2Ax peptides.

- Successful application of the assay to cytoplasmic and nuclear fractions derived from senescent and self-renewing adipose-derived mesenchymal stem cells.

It has been shown that un resolves persistent DNA damage occurs upon senescence. This consistent with the apparent increase in ser 139 phosphorylated H2Ax in our data. In order to understand the importance of chromatin dynamics in aging, we next plan to investigate the changes in the chromatin-associated histone chaperones and chromatin-remodeling complexes upon replicative senescence of human adult mesenchymal stem cells. It is known that both core histone chaperones and ATP-utilizing motor proteins incorporated into histone remodeling complexes can interact with repair factors and transmit signaling to cell cycle control machinery. Several distinct histone chaperones, such as CAP1 (chromatin assembly factor-1), ADP (anti-silencing function-1), NAP1 (nucleosome assembly protein-1) and HIRA (histone regulatory protein A) participate in the deposition of the histones onto the DNA and/or mediate the translocation of core histones from the cytoplasm to the nucleus.

**References**