A multi-modal proteomics strategy for characterizing in vitro p53 modforms
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INTRODUCTION

Our primary objective was to apply a combined strategy of denaturing top-down MS, bottom-up MS, and multiple reaction monitoring (MRM) MS to identify and characterize the most abundant modforms (proteoforms) bearing enzymatically reversible post-translational modifications (PTMs), and to enumerate the number of possible modforms and determine the actual modform distribution within the representative populations.

Endogenous human p53 is extensively modified

Endogenous p53 PTMs most likely comprise a combinatorial code

Proposed analysis of endogenous p53 modforms by top-down mass spectrometry

We hypothesized that the examination of endogenous and recombinant human p53 by top-down MS (TDMS) would enable the generation of a comprehensive modform profile, comprising all modforms of p53 present within a specific cellular context, and the comprehensive modulation of the conditional regulatory networks underlying observed modforms of p53. To that end, we have developed a method by which top-down acquisition of intact, recombinant endogenous human p53 can be performed, and analyzed for the presence of PTMs by a combined strategy of high-resolution tandem MS. This approach promises to provide a new method for studying PTMs that enables the analysis and characterization of endogenous p53 and recombinant human p53 in a broad range of cellular contexts, including multiple disease response pathways (e.g. DNA damage and stress response systems). This will allow for the identification of novel interactions and modulations of these pathways, as well as the elucidation of the complex PTM landscape of p53 in different cellular contexts.

We will develop a network of linear equations to accurately describe both p53 modforms and their relative abundances within a population indicated by empirical top-down, bottom-up, and MRM MS data. We will then determine if the observed modform distribution is consistent with the previously characterized distribution, allowing for the detection of novel PTMs and the elucidation of the complex PTM landscape of p53 in different cellular contexts.

RESULTS

Characterization of C-Terminal p53 modforms by combined top down and bottom-up mass spectrometry

Analysis of model p53 proteoforms by Multiple Reaction Monitoring (MRM)

Analysis of unmodified p53 by denaturing top-down mass spectrometry

Analysis of representative p53 modforms by denaturing top-down mass spectrometry

Analysis of model p53 proteoforms by Multiple Reaction Monitoring (MRM)

Example Chromatogram (TSQ Quantiva):

Example Coverage Map (TSQ Quantiva):

Example Chromatogram (21T FT-ICR):

Example isolated MS1 spectrum of the 44+ charge state of intact and unmodified rp53 acquired at 300,000 FTRP on an LTQ ion trap.

Top: Example isolated MS1 spectrum of the 13.2 kDa rp53 C-terminal fragment (21T FT-ICR): Bottom: Example isolated MS1 spectrum of the 10.7 kDa rp53 C-terminal fragment (21T FT-ICR):

Zoomed-in view of the 18+ charge state of the 13.2 kDa rp53 C-terminal fragment acquired at 150,000 FTRP on an LTQ ion trap. Example Coverage Map (TSQ Quantiva):

Example peptide peaks from liquid chromatography electrophoresis and fragmentation by top-down MS.

Example peptide peaks from liquid chromatography electrophoresis and fragmentation by bottom-up MS.

Example peptide peaks from liquid chromatography electrophoresis and fragmentation by both top-down and bottom-up MS.

Future Directions

Model to Determine Actual Modform Distributions:

Comparison of Endogenous Modform Distributions:

We will compare the modform distributions between the in vitro and in vivo modform distributions, in order to determine whether it is the differences in specific PTMs or the distribution of PTMs that cause distinct dynamic and phenotypic outcomes.

METHODS

Analysis of representative p53 modforms by denaturing top-down mass spectrometry

Experimental strategy:

Analysis of unmodified p53 by denaturing top-down mass spectrometry

Analysis of model p53 proteoforms by Multiple Reaction Monitoring (MRM)

RESULTS

Analysis of unmodified p53 by denaturing top-down mass spectrometry

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