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High Sensitivity Top-Down Proteomics Reveals Heterogeneous Proteoform Profiles Amongst Various Single Skeletal Muscle Cells

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Speaker: Jake Melby, Ph.D., Department of Chemistry University of Wisconsin-Madison Wisconsin

Introduction

Single muscle fibers (SMFs) are multinucleated single cells that can be classified into fast- and slow-twitch fibers. Post-translational modifications (PTMs) and isoforms of myofilament proteoforms within SMFs have profound effects on functional properties. To better understand how PTMs/isoforms affect fiber function, it is important to analyze myofilament proteoforms within SMFs instead of their constituent muscles, which contain a heterogeneous mixture of fast/slow-twitch SMFs, connective tissue, and blood vessels. Due to the inherent challenges associated with top-down proteomics, conventional methods extract proteoforms from large pieces of muscle, which convolutes the relationship between proteoforms and SMF function. Therefore, we have developed a highly sensitive top-down proteomics strategy for the analysis of proteoforms from SMFs, which revealed distinct proteoform profiles compared to tissue.

Methods

SMFs were dissected from rat plantaris (PLN) hindlimb muscle in relaxation buffer containing protease and phosphatase inhibitors. To minimize sample losses, a one-vessel extraction method was used to extract myofilament proteoforms from SMFs (n=6) using MS-compatible solvents and freeze-thaw lysis. In addition, a previously reported, acidic extraction for the sarcomere subproteome from PLN tissue (n=3) was performed to compare the proteoform results from tissue to SMFs. Online liquid chromatography-MS (LC-MS) was performed using a Thermo MAbPac Capillary Reversed Phase column with the Newomics microflow-nanoelectrospray ionization (MnESI) source housing a 10 µm inner diameter M3 multinozzle emitter. MS data were acquired using a Bruker maXis II quadrupole time-of-flight mass spectrometer. Bruker DataAnalysis and MASH Explorer software were used for data processing.

Preliminary Data

Our highly sensitive top-down proteomics method successfully extracted and characterized contractile proteoforms within single muscle cells. When analyzing the data obtained from the PLN

SMFs, almost all of the contractile proteoforms were found to be in the fast-twitch isoform. For example, tropomyosin (α-Tpm), myosin light chains (MLC-1F, MLC-2F, and MLC-3F), and the troponin complex (fsTnI, fsTnT and fsTnC), were successfully analyzed by high sensitivity top-down MS. However, one of the PLN SMF samples did contain slow-twitch isoforms, such as MLC-2S, confirmed by tandem MS. Additionally, each SMF contained different relative abundance of contractile proteoforms and the level of phosphorylation (Ptotal) was highly heterogeneous amongst each PLN SMF sample. The data obtained from the PLN tissue samples had stark differences when compared to the PLN SMF data. In addition to the fast-twitch isoforms found in the SMF data, there were also several slow-twitch isoforms detected in the PLN tissue data, including MLC-1S, MLC-2S, ssTnC, and β-Tpm. The relative abundance of contractile proteoforms as well as the Ptotal calculations were highly reproducible across the biological replicates. There were several proteins found in the sample pertaining to blood vessels (myoglobin), nerves, and connective tissue that were not found in the SMF extracts. Collectively, the tissue data represents an average of the constituent slow- and fast-twitch SMF and other components, such as blood vessels, found in skeletal muscle. The overall results illustrate fiber-to-fiber and fiber-to-tissue proteoform differences due to high sensitivity top-down proteomics. Performing top-down proteomics at the single fiber level provides a more accurate assessment of fiber-specific PTMs/isoform compared to bulk tissue analysis, which can reveal biological phenomena occurring at the single fiber level. We plan to integrate this method with fiber contractile measurement to aid in linking proteoforms with function to better understand muscular diseases.

Novel Aspect

First high sensitivity top-down proteomics of multinucleated single muscle cells for the characterization of myofilament protein PTMs and isoforms.