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Perspectives

PERSPECTIVES

Expanding the MuRF1 Universe with Quantitative Ubiquitylomics

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A Perspective on *"Identification of the MuRF1 Skeletal Muscle Ubiquitylome Through Quantitative Proteomics"*

It is now getting close to 50 yr since the small, highly conserved protein, ubiquitin, (a.k.a. ubiquitous immunopoietic polypeptide; UBIP) was first isolated in 1975 by Goldstein et al. who, despite not yet knowing its function, speculated "…we can infer that the function of UBIP is an integral feature of living cells".[1] In 1977, a soluble, non-lysosomal, ATP-dependent proteolytic system, now known as the proteosome, was identified by Etlinger and Goldberg for degrading abnormal proteins for which the authors suggested "…may also be involved in hydrolysis of normal cell proteins".[2] By 1980, Hershko et al. demonstrated that the covalent conjugation of a protein with chains of multiple ubiquitin molecules targeted that protein for ATP-dependant, non-lysosomal-mediated, proteolysis,[3] while in 1983, the same group purified the three sequential enzymes of the ubiquitin–protein ligase system (ubiquitin-activating enzyme, ubiquitin-conjugating enzyme, and ubiquitin ligase), and introduced the 'E3' nomenclature (ie the third factor eluted via affinity chromatography) for ubiquitin ligases.[4] This work eventually led to the awarding of the Nobel Prize in Chemistry (https://www.nobelprize.org/prizes/chemistry/2004/summary/) in recognition of the fundamental importance of ubiquitin–proteasome system for the normal cellular function.

In the context of skeletal muscle, fast forward to 2001 and a previously identified muscle-specific RING finger protein, MuRF1 (a.k.a SRMZ and Trim63), was shown by Bodine et al. to be upregulated in muscles from mice subjected to different models of muscle atrophy and to have E3 ubiquitin ligase activity.[5] Moreover, MuRF1 knockout mice were resistant to denervation-induced muscle atrophy.[5] Combined, this seminal study strongly suggested that MuRF1 played an important role in the regulation of ubiquitin proteasome-mediated protein degradation in skeletal muscle and that its upregulation may promote muscle atrophy (although this was not demonstrated in that study). This study triggered tremendous interest in the role of the ubiquitin–protein ligase system and, in particular, E3 ligases in the regulation of skeletal muscle mass/function, spawning a huge number of descriptive studies detailing changes in MuRF1 mRNA/protein expression (and other E3 ligases), in a range of different models of muscle adaptation in health and various disease states. In addition, more mechanistic studies identified transcriptional regulators of MuRF1 expression (eg FoxO3, Smad3, NF-κB, and myogenin), putative MuRF1-associated E2 ubiquitin conjugating enzymes (eg E2E1, E2EG1, E2J1, E2J2, and E2L3), and proteins that interact with MuRF1, some of which have been identified as MuRF1 substrates (eg myosin heavy chains, myosin-binding protein C, myosin light chains, telethonin, titin, and so on[6]; however, a more comprehensive screen for putative MuRF1 substrates had been lacking.

In this issue of *Function*, Baehr et al. combined transient overexpression MuRF1 in mouse skeletal muscle with quantitative proteomics/ubiquitylomics in an attempt to gain a more complete picture of potential MuRF1 substrates.[7] Firstly, they found that, unlike data derived from a transgenic skeletal muscle-specific MuRF1 overexpressing mouse model,[8]

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transient MuRF1 overexpression was sufficient to induce a reduction in muscle mass and muscle fiber cross-sectional area and that this was associated with increased protein ubiquitylation.[7] Furthermore, this atrophic effect required a functional RING domain, presumable to facilitate interaction with E2 ubiquitin conjugating enzymes.[7] Importantly, label-free quantitative mass spectrometry was then used to detect the di-glycine (diGly) tag that remains covalently attached to lysine residues after trypsin digestion which represents a remnant of ubiquitylation; although the presence of a diGly tag does not discriminate between the different types of ubiquitylation (ie monoubiquitylation vs polyubiquitylation and branched vs linear polyubiquitin chains). Using this approach, the authors detected 963 ubiquitylation sites, from 250 proteins, with a single ubiquitylation site detected on 143 proteins, while the remaining proteins had multiple ubiquitylation sites. Of these proteins, MuRF1 overexpression upregulated ubiquitylation at 153 lysine residues on 45 proteins and downregulated ubiquitylation at 16 sites on 11 proteins. While some previously identified MuRF1 substrates were found to have increased ubiquitylation, including titin, most of the proteins with increased ubiquitylation have not previously been associated with MuRF1. This analysis represents a major enlargement of the pool of potential MuRF1 substrates. Further validation studies are required to separate bona fide MuRF1 substrates from substrates of other E3 ligases that may have been upregulated in response to MuRF1 overexpression, and to determine whether ubiquitylation leads to target protein degradation or to changes in protein function, interactions, and/or localization.

Another reason that this study is significant is that it is one of only a few studies to have employed mass spectrometry-based ubiquitylomics with a model of muscle atrophy, including three previous studies that examined denervation,[9] immobilization,[10] and overexpression of another muscle-specific E3 ligase, ASB2β.[11] Importantly, these studies allow us to begin to compare changes to the ubiquitylome in different models of muscle atrophy to see if there is any sign of a conserved signature or whether there are model-specific differences in the regulation of protein ubiquitylation. For example, in this study by Baehr et al., the protein with the highest number of differentially regulated diGly-tagged lysine residues was the giant structural protein, titin, which had significantly increased ubiquitylation at 63 sites[7]; however, only one of these titin lysine residues had a significantly increased diGly signature with ASB2β overexpression,[11] and only three of these lysine residues had increased diGly with denervation.[9] Moreover, none of the titin lysine residues that had increased diGly with MuRF1 overexpression were detected in the immobilization study,[10] an interesting observation given that MuRF1 expression is increased with immobilization.[6] Notwithstanding differences in the technologies and time points used in these studies, these comparisons suggest the possibility of atrophy model-dependent differences in protein ubiquitylation that warrants further investigation of individual protein ubiquitylation in these, and additional, models of atrophy.

Overall, this new study by Baehr et al. expands our knowledge of the role of MuRF1 in skeletal muscle atrophy and of MuRF1-medated changes to the skeletal muscle ubiquitylome. Moreover, this study continues to extend the field of the role of ubiquitylation in the regulation of skeletal muscle mass/function that ultimately began almost 50 yr ago when Goldstein et al. first isolated ubiquitin.

Conflict of Interest Statement

None declared.

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