Editorial title:
Putting the stress on UFM1:
Protein Ufmylation as a Novel Regulator of ER Stress in Cardiac Health and Disease

Authors/titles:
James D. Sutherland, PhD & Rosa Barrio, PhD

Institutional affiliation:
CIC bioGUNE, Bizkaia Technology Park, Building 801A, 48160 Derio, Spain

Defects in protein homeostasis (also known as proteostasis) are intrinsically linked to age-related decline of cardiac function and likely contribute to cardiomyopathies. Protein-folding chaperones and protein quality control systems work overtime in the high stress cardiac environment, responding to wear and tear. Tight regulation of the pathway components is often controlled by post-translational modifications (PTMs). In addition to compact PTMs such as phosphorylation, bulkier PTMs involve ubiquitin (Ub) and other small ubiquitin-like (UbL) proteins, which are covalently conjugated to target proteins. These modifications can modify stability, localization, and function of the target protein. Ufm1 (ubiquitin-fold modifier 1) is a less-studied UbL, but modification by Ufm1 (ufmylation [1]) is emerging as an important mediator of the endoplasmic reticulum (ER) stress response, which is activated in cardiomyocytes during heart failure. Focusing on Ufl1 (Ufm1-ligase 1), one of the enzymes that mediate ufmylation, the report from Huabo Su and colleagues [2] provides strong evidence that Ufl1 has a cardioprotective role and points to the ufmylation pathway as a potential target for pharmacological intervention for some cardiomyopathies.

Ub and UbLs share a common biology in the way they are added to targets and recycled in the cell, but each class tends to have specialized roles [3]. They are varied in their individual protein sequences, with Ub/UbLs sharing a common structural fold as revealed by crystallographic and NMR-based protein studies. After synthesis as a pro-protein, Ub/UbLs are cleaved at the C-terminus by a protease, a prerequisite for entering the conjugation cycle. The cleaved Ub/UbL is engaged by an activation enzyme (generically termed E1, or “enzyme 1”). Via this Ub-E1 intermediate, the Ub/Ubl is then passed to a conjugating enzyme (E2 or “enzyme 2”). In some cases, the E2 can engage directly with target proteins, and the Ub/UbL is conjugated by its C-terminus to the targets, usually to a lysine. In most cases, however, the Ub-E2 intermediate engages with a ligating enzyme (or E3 ligase; “enzyme 3”). The structure of the E3 may vary greatly, from being a single protein to a multi-protein complex, but in general, the E3 serves to enhance delivery of the mature Ub/Ubl to a lysine on specific target proteins. Certain conserved protein domains are characteristic of E3 ligases (e.g. RING or HECT domains), and by this measure, it is estimated that >600 E3 Ub/UbL ligases are encoded by the human genome. Other E3 ligases have been described that lack these domains but may function as scaffolding adaptors to bridge the E2-Ub/Ubl and targets (Ufl1, featured in Li et al. [2], is one example). Ub/UbL PTMs vastly increase the complexity of the human proteome and require exquisite regulation to allow cells to cycle, survive, and respond to signals and stress.

Correct regulation of proteostasis underlies both healthy and diseased/damaged heart function. This complex task balances protein synthesis, folding, and degradation, with the latter relying on multiple systems (autophagy, calpains, proteasomes [4, 5]). Specifically, contributions to cardiac proteostasis by the Ub-proteasome system have been extensively reviewed [6, 7]. Cardiac roles for modifications by other UbLs (NEDD8, SUMO) have also been described [8, 9]. Many of the studies defining cardiac roles for Ub/UbL modifications rely on either classical or conditional knockout mice, with the

latter allowing heart-specific deletion of the genes encoding the UbL or its associated pathway enzymes. These precise genetic studies allow molecular, histological, and physiological analysis of the heart in experimental animals. When coupled with surgical methods to induce heart stress or with drug administration or both, such studies can reveal promising targets and therapies for future clinical trials.

Ufmylation was discovered by chance in a search for proteins implicated in autophagy [10], and like ubiquitylation, it occurs through a multi-enzyme cascade (Figure 1). The E1 activating enzyme Uba5 was identified first, and further biochemical and proteomic analysis revealed Ufm1 as the novel UbL that is activated by Uba5. The same study also discovered the dedicated E2 enzyme, Ufc1, responsible for conjugation of Ufm1 to target proteins. Later studies uncovered Ufl1, a Ufm1 E3 ligase, as an interactor of both Ufm1 and the E2 Ufc1 [11]. The search for proteases that were capable of processing pro-Ufm1 and that could remove and recycle Ufm1 from target proteins yielded two related proteins, Ufsp1 and Ufsp2 [12]. All of these proteins show close association to the ER, and functional studies (including the present report) point to specialized roles for ufmylation in regulating ER-related stress. The ER is a major site of protein quality control, housing the synthesis machinery for proteins destined to be secreted or membrane-trafficked within the cell, as well as chaperones to assist protein folding and three separate systems (PERK, ATF6, IRE1) that mediate the unfolded protein response (UPR). A number of ufmylated target proteins have been identified, although these are few in number compared to those modified by Ub or SUMO. One ufmylated target, Ufbp1 (also known as Ddrgk1), warrants particular mention since it is required for efficient ufmylation of other targets and may act as a co-factor for the Ufl1 E3 ligase. Loss-of-function classical knockouts in mice (Uba5, Ufl1, or Ufbp1) have revealed an essential role for ufmylation in erythroid development and lead to embryonic lethality [1] . Tissue-specific knockouts can be utilized to reveal roles later in development, exemplified by Li et al. [2] (this issue). This approach has uncovered a novel role for Ufl1 in mouse cardiac health.

Their study begins with the observation that, although ufmylation of major target proteins in the heart appears to increase over time (from 1 day to 1 year-old), levels of Ufl1 E3 ligase seem to decrease. This may seem counterintuitive, but possible explanations may lie in altered levels or activities of other ufmylation mediators (Uba5, Ufc1, Ufbp1, Ufsp1/2). Upon surgically induced cardiac hypertrophy in mice, levels of both ufmylation and Ufl1 increased. Levels of Ufl1 decreased during induced ischemia/reperfusion, likely due to cardiomyocyte death. Samples from patients with dilated cardiomyopathy also showed reduced Ufl1 levels, all leading to the hypothesis that ufmylation might be a key player in cardiac stress response. To address this, the authors used a conditional mouse mutant to remove Ufl1 from the cardiomyocyte population, in order to see how this affected both normal heart development as well as response to heart stress and injury.

While Ufl1 levels were significantly reduced in whole heart extracts from knockouts, detectable Ufl1 and ufmylation still remained, suggesting that genetic removal was not complete, or that non-cardiomyocyte cells contribute to residual activity. Alternatively, as seen with SUMOylation, perhaps ufmylation of some targets does not require an E3 ligase, or perhaps another uncharacterized Ufm1 E3 ligase acts redundantly to Ufl1 (Figure 1). Still, reduction of Ufl1 was sufficient to induce cardiac remodeling and progressive functional deterioration as measured by echography, histology, and changes in gene expression. Using induced cardiac hypertrophy, $Ufl1^{CKO}$ mice were unable to make compensatory changes and developed heart failure, with increased fibrosis and decreased contractility, suggesting that Ufl1 has a cardioprotective role.

Turning to molecular mechanisms, comparative analysis of transcriptomes from control and Ufl1^{CKO} mice pointed to ER dysfunction when Ufl1 was reduced. Indeed, as reported previously for cells and other organ systems, loss of Ufl1 and ufmylation in the heart leads to increased ER stress, evident as an increase in stress response chaperones and changes in ER ultrastructure. Induced cardiac hypertrophy also induced stress chaperones, but the same treatment in Ufl1^{CKO} mice led to even higher levels. Using primary cardiomyocytes from rat and drugs to induce ER stress, the authors show that at least one of the three UPR pathways (PERK) was affected by Ufl1 reduction. Using a previously reported strategy [13], administration of tauroursodeoxycholic acid (TUDCA), a chemical chaperone known to alleviate ER stress, was able to reduce the cardiac enlargement and ER stress-induced cell death seen in Ufl1^{CKO} mice with surgically-induced cardiac hypertrophy. Overall, the results convincingly show that Ufl1 and ER stress regulation are tightly connected and that ufmylation may be a druggable cardioprotective pathway.

Although TUDCA is being testing in clinical trials, including some focused on cardiac issues (e.g. www.clinicaltrials.gov NCT01855360), a treatment more focused on Ufl1 and the ufmylation pathway may yield less secondary effects. Given the current push to exploit E3 ligases and deubiquitinases as druggable targets [14, 15], the ufmylation pathway will certainly be explored and new drug candidates will likely emerge in the upcoming years. Li et al. suggests that activation of ufmylation would be beneficial in the case of some cardiomyopathies and perhaps age-related decline of cardiac health. A better understanding of how Ufl1 interacts with targets for ufmylation and how Ufsp1 (the potential Ufl1 cofactor; Figure 1) contributes to the E3 activity of Ufl1 may lead to new ideas on how to find or design activating compounds. Activators of Uba5 and/or Ufc1 could also lead to overall increases of ufmylation. Conversely, a search for inhibitors of Ufm1 proteases may identify a compound capable to enriching for ufmylated proteins. A recent report shows that loss of Ufsp2 in cells leads to a dramatic increase in ufmylated targets [16], suggesting that inhibitors may yield the desired effect. Since heart failure is a leading cause of morbidity and mortality, especially among middle-aged and older adults, this important study reveals ufmylation as a promising pathway to explore for therapeutic benefits in cardiomyopathies, and likely other ER stress-based illnesses.

Sources of Funding

Dr. Sutherland and Dr. Barrio are funded by BFU2017- 84653-P (MINECO/FEDER, EU), the Severo Ochoa Excellence Program (SEV-2016- 0644), the UbiCODE program (765445, EU) and PROTEOSTASIS (COST BM1307, EU). Additional support was provided by the Department of Industry, Tourism, and Trade of the Government of the Autonomous Community of the Basque Country (Elkartek Research Programs) and by the Innovation Technology Department of the Bizkaia County.

Disclosures

None.

Footnotes

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

Correspondence to James D. Sutherland, PhD;
CIC bioGUNE, Technology Park of Bizkaia, Bldg 801A, Derio, SPAIN 48160

E-mail: jsutherland@cicbiogune.es

References

1. Wei Y and Xu X. UFMylation: A Unique & Fashionable Modification for Life. *Genomics Proteomics Bioinformatics*. 2016;14:140-146.
2. Li J, Yue G, Ma W, Zhang A, Zou J, Cai Y, Tang X, Wang J, Liu J, Li H and Su H. The Ufm1-Specific Ligase Ufl1 Regulates Endoplasmic Reticulum Homeostasis and Protects against Heart Failure. *Circ Heart Fail*. 2018;??:??
3. van der Veen AG and Ploegh HL. Ubiquitin-like proteins. *Annu Rev Biochem*. 2012;81:323-57.
4. Henning RH and Brundel B. Proteostasis in cardiac health and disease. *Nat Rev Cardiol*. 2017;14:637-653.
5. Sciarretta S, Maejima Y, Zablocki D and Sadoshima J. The Role of Autophagy in the Heart. *Annu Rev Physiol*. 2018;80:1-26.
6. Gilda JE and Gomes AV. Proteasome dysfunction in cardiomyopathies. *J Physiol*. 2017;595:4051-4071.
7. Day SM. The ubiquitin proteasome system in human cardiomyopathies and heart failure. *Am J Physiol Heart Circ Physiol*. 2013;304:H1283-93.
8. Kandala S, Kim IM and Su H. Neddylation and deneddylation in cardiac biology. *Am J Cardiovasc Dis*. 2014;4:140-58.
9. Da Silva-Ferrada E, Ribeiro-Rodrigues TM, Rodriguez MS and Girao H. Proteostasis and SUMO in the heart. *Int J Biochem Cell Biol*. 2016;79:443-450.
10. Komatsu M, Chiba T, Tatsumi K, Iemura S, Tanida I, Okazaki N, Ueno T, Kominami E, Natsume T and Tanaka K. A novel protein-conjugating system for Ufm1, a ubiquitin-fold modifier. *EMBO J*. 2004;23:1977-86.
11. Tatsumi K, Sou YS, Tada N, Nakamura E, Iemura S, Natsume T, Kang SH, Chung CH, Kasahara M, Kominami E, Yamamoto M, Tanaka K and Komatsu M. A novel type of E3 ligase for the Ufm1 conjugation system. *J Biol Chem*. 2010;285:5417-27.
12. Kang SH, Kim GR, Seong M, Baek SH, Seol JH, Bang OS, Ovaa H, Tatsumi K, Komatsu M, Tanaka K and Chung CH. Two novel ubiquitin-fold modifier 1 (Ufm1)-specific proteases, UfSP1 and UfSP2. *J Biol Chem*. 2007;282:5256-62.
13. Rani S, Sreenivasaiah PK, Kim JO, Lee MY, Kang WS, Kim YS, Ahn Y, Park WJ, Cho C and Kim DH. Tauroursodeoxycholic acid (TUDCA) attenuates pressure overload-induced cardiac remodeling by reducing endoplasmic reticulum stress. *PLoS One*. 2017;12:e0176071.
14. Harrigan JA, Jacq X, Martin NM and Jackson SP. Deubiquitylating enzymes and drug discovery: emerging opportunities. *Nat Rev Drug Discov*. 2018;17:57-78.
15. Huang X and Dixit VM. Drugging the undruggables: exploring the ubiquitin system for drug development. *Cell Res*. 2016;26:484-98.
16. Ishimura R, Obata M, Kageyama S, Daniel J, Tanaka K and Komatsu M. A novel approach to assess the ubiquitin-fold modifier 1-system in cells. *FEBS Lett*. 2017;591:196-204.

Figure legend:

Figure 1. Features of the ufmylation cycle, a key pathway for endoplasmic reticulum-based (ER) stress response in cardiac development and disease. UFM1 is conjugated to substrates through a multi-enzyme cascade. After proteolytic maturation, UFM1 is passed via three enzymes (activating E1, conjugating E2 and ligating E3) as it attaches to substrates. Specific proteases remove and recycle Ufm1. Li et al. [2] demonstrate that Ufl1, a Ufm1 E3 ligase, is cardioprotective in both physiological and pathological conditions. How ufmylation influences substrates, whether E3 ligases are obligatory, whether additional E3 ligases exist, and how the Ufbp2 cofactor affects E3 activity are still open questions for future investigation. Even so, the ufmylation pathway may offer unique drug targets for cardiac and other diseases that feature ER stress response as cause or consequence of the pathology.