Disruption of valosin-containing protein activity causes cardiomyopathy and reveals pleiotropic functions in cardiac homeostasis

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Received for publication, January 16, 2019, and in revised form, April 8, 2019. Published, Papers in Press, April 21, 2019, DOI 10.1074/jbc.RA119.007585

This work was supported by NIH grants F32HL124698 and K99HL136695 (to M. J. B.) and R01HL105924 (to J. D. M.) and grants from the Howard Hughes Medical Institute, 240 Albert Sabin Way, MLC7020, the Howard Hughes Medical Institute, 240 Albert Sabin Way, MLC7020, and the Howard Hughes Medical Institute. The authors declare that they have no conflicts of interest. The authors and does not necessarily represent the official views of the authors and does not necessarily represent the official views of the National Institutes of Health.

Valosin-containing protein (VCP), also known as p97, is an ATPase with diverse cellular functions, although the most highly characterized is targeting of misfolded or aggregated proteins to degradation pathways, including the endoplasmic reticulum–associated degradation (ERAD) pathway. However, how VCP functions in the heart has not been carefully examined despite the fact that human mutations in VCP cause Paget disease of bone and frontotemporal dementia (IBMPFD), a degenerative multisystem proteinopathy that causes pathology of the skeletal muscle, brain, bone, and heart. IBMPFD is characterized by progressive muscle weakness that presents in adulthood and can ultimately include heart failure, dementia, and abnormal bone growth (1–4). VCP forms a barrel-shaped homohexamer that functions as an ATPase through the action of its D1 and D2 domains, whereas its N-terminal domain recruits VCP to different subcellular locations (5–10). VCP has been ascribed an array of cellular functions, including protein quality control (7, 11–16), membrane fusion (17), chromatin remodeling (18), disassembly of the DNA helicase complex (19, 20), DNA repair (21), formation of the endoplasmic reticulum (ER) (22) and nuclear envelope (23), and regulation of RNA stability (24). However, the best-characterized molecular function for VCP is providing energy to extract terminally misfolded polypeptides from the ER into the cytosol for degradation by the ubiquitin proteasome system (UPS) in a process termed ER-associated degradation (ERAD) (7, 11–14, 16). VCP additionally functions in nuclear protein quality control in a pathway analogous to ERAD at the inner nuclear membrane (INM) (25, 26).

VCP also directly associates with the large ribosomal subunit (27–29) and functions in ribosome-associated degradation in yeast to release aberrant translated polypeptides from ribosomes for proteasomal degradation (27, 28, 30). A large portion of cellular energy is expended to assemble, maintain, and degrade the upwards of 10 million ribosomes present in a mammalian cell, which requires the action of diverse nonribo-
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Figure 1. Impairment of cardiomyocyte VCP activity causes cardiomyopathy. A, schematic of the binary transgenic system used to induce heart-specific expression of WT VCP or an ATPase-deficient VCP mutant (VCP^K524A). B, Western blot of cardiac lysates for VCP in DTg mice overexpressing WT or K524A mutant VCP and single transgenic controls at 2 months of age. GAPDH was used as a sample processing and loading control. C, heart weight-to-body weight ratios at 6 months of age in the indicated genotypes of mice. D, gross morphology (top) and histology (H&E, bottom) of DTgVCP^K524A and tTA control hearts at 9 months of age. Scale bars = 1 mm. E–G, cardiac structure and function were evaluated at various ages in the indicated groups of mice by echocardiography to measure left ventricular inner chamber dimension in diastole (LVIDd, E), left ventricular inner chamber diameter in systole (LVIDs, F), and fractional shortening percentage (FS, G) in mouse hearts at the indicated age in months. The number of mice analyzed is shown in the columns in C and E–G. *p < 0.05 compared with tTA controls using one-way ANOVA with Tukey’s post hoc test.

VCP is abundantly expressed in the heart (34), and IBMpFD includes cardiomyopathy and heart failure (1–4). However, the pathways and processes that are regulated by VCP in cardiomyocytes are not known. To address this, we generated heart-specific transgenic mice overexpressing WT VCP or a VCP mutant with impaired ATPase activity (VCP^K524A) (8). Although overexpression of WT VCP is without noticeable effect, transgenic VCP^K524A mice develop cardiomyopathy, up-regulate the ERAD machinery, and accumulate ubiquitinated proteins in the heart. Moreover, cardiomyocyte nuclear morphology is disrupted in VCP^K524A-overexpressing hearts, suggesting roles of VCP in maintaining the integrity of cardiomyocyte nuclei. Surprisingly, proteomics identified predominant interactions of VCP with ribosomal proteins, ribosome-associated proteins, and RBPs. Further investigation revealed accumulation and aggregation of large ribosomal subunit proteins in VCP-mutant hearts, suggesting underappreciated functions of VCP in ribostasis that may also contribute to IBMpFD in humans. Taken together, we have uncovered multiple protein and RNA quality control pathways through which VCP functions in cardiomyocytes to maintain cardiac homeostasis.

Results

Inhibition of VCP activity in cardiomyocytes causes cardiomyopathy

To investigate the cardiac functions of VCP, we generated transgenic mice with cardiomyocyte-specific overexpression of VCP. We utilized a bigenic transgene system that requires expression of both the tetracycline transactivator (tTA) transgene and the VCP transgene that contains the tetracycline operator within the α-myosin heavy chain (α-MHC) promoter to produce tetracycline- or doxycycline-repressible expression in cardiomyocytes (35) (Fig. 1A). The presence of both transgenes is referred to as double-transgenic (DTg). In addition to transgenic mice overexpressing WT VCP, we generated transgenic mice overexpressing a VCP^K524A mutant (Fig. 1A) to interrogate the effects of impaired VCP activity in the heart. Mutation of lysine 524 within the D2 ATPase domain of VCP to alanine results in dramatically reduced ATPase activity and produces a dominant-negative mutant that is deficient in segregase and protein complex remodeling functions (8). As expected, the hearts of WT or mutant VCP DTg mice maintained on normal lab chow in the absence of tetracycline or doxycycline (the bigenic transgene system is fully induced) had significantly increased VCP protein expression (Fig. 1B). WT and K524A mutant VCP transgenic mice showed no cardiac hypertrophy at 6 months of age (Fig. 1C). However, analysis of gross morphology and histology at 9 months of age revealed cardiac enlargement and atrial dilation in DTgVCP^K524A mice but not the WT (Fig. 1D). Cardiac structure and function of transgenic mice were evaluated by echocardiography, demonstrating normal cardiac morphology and function in mice over-expressing WT or mutant VCP in the heart through 6 months of age (Fig. 1, E–G), but by 8–10 months of age, DTgVCP^K524A mice developed systolic dysfunction, whereas aged DTgVCP mice remained normal (Fig. 1, F and G). Cardiomyopathy in aged DTgVCP^K524A mice occurs in the absence of significant somal proteins, including many ATPases, RNA helicases, and RNA-binding proteins (RBPs) (31–33). However, specific functions for VCP in these processes have not been established.

The presence of both transgenes is referred to as double-transgenic (DTg). In addition to transgenic mice overexpressing WT VCP, we generated transgenic mice overexpressing a VCP^K524A mutant (Fig. 1A) to interrogate the effects of impaired VCP activity in the heart.
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left ventricular hypertrophy or remodeling (Fig. 1, C–G, and Fig. S1, A and B). Thus, inhibition of VCP activity ultimately has a negative effect on cardiac function, suggesting important roles of VCP in cardiac homeostasis.

To further examine the pathologic effects of disrupting VCP ATPase activity in the heart, we performed transverse aortic constriction (TAC) to surgically induce pressure overload hypertrophy in VCP and VCPK524A transgenic mice at 8–10 weeks of age. After 4 weeks of pressure overload, mice overexpressing VCPK524A exhibited a substantial decline in cardiac function compared with tTA controls and mice with heart-specific overexpression of WT VCP (Fig. 2, A–C). By 8 weeks following TAC surgery, DTgVCPK524A mice developed dilated cardiomyopathy, characterized by diminished cardiac functional performance, whereas tTA controls and DTgVCP mice did not undergo significant ventricular remodeling and had a relatively preserved cardiac functional response to pressure overload (Fig. 2, A–C). Histological evaluation confirmed ventricular dilation and revealed prominent dilation of the left atrium in DTgVCPK524A mice (Fig. 2D), indicating increased sensitivity to pressure overload–induced cardiac dysfunction and remodeling when VCP ATPase activity is defective, compared with tTA control mice. Taken together, these data demonstrate that disruption of VCP ATPase activity causes age-dependent cardiomyopathy and hastens cardiac disease in response to pressure overload.

VCP activity is critical for cardiac proteostasis and ERAD

VCP underlies protein quality control, which commonly involves degradation of proteins by the UPS, including an indispensable role in the ERAD pathway, which removes misfolded proteins from the ER for UPS-mediated degradation in the cytoplasm (7, 11–14, 16). Hearts overexpressing VCPK524A but not WT VCP had a significant elevation of ubiquitinated proteins in the soluble fraction (Fig. 3, A and B), indicating that inhibition of cardiac VCP activity results in defective clearance of proteins targeted for degradation by the UPS. Importantly, the accumulation of ubiquitinated proteins in DTgVCPK524A hearts (Fig. 3, A and B) preceded cardiomyopathy, suggesting that proteotoxicity contributes to disease caused by inhibition of VCP ATPase activity.

Next we evaluated the ERAD pathway in transgenic hearts with manipulated VCP activity given the build-up of ubiquitinated proteins in DTg mutant hearts (Fig. 3, A and B) and given the known importance of ERAD in cardiac physiology (36). We performed Western blotting for various components of the ERAD pathway and ER-resident chaperones involved in protein folding and quality control. Sel1L, an ER membrane–embedded adaptor required for the dislocation of improperly folded proteins in the ER for degradation by ERAD (37–40), was up-regulated in hearts overexpressing either VCP or VCPK524A compared with tTA controls (Fig. 3, C and D), indicating that VCP overexpression stabilizes an ERAD complex at the cardiomyocyte ER membrane. Homocysteine-induced ER protein (Herp), another crucial ER membrane adaptor for ERAD (41), and OS-9, an ER luminal lectin that binds misfolded glycoproteins in the ER for recognition by the ERAD complex (41, 42), were up-regulated specifically in hearts overexpressing the VCPK524A mutant (Fig. 3, C and D). Importantly, the mRNA levels of the ERAD genes Sel1L, OS9, and Herpud1 (encodes Herp) were not up-regulated, and some were even down-regulated in VCP mutant–overexpressing hearts (Fig. S2), indicating that the increased protein levels of ERAD factors observed in VCP-mutant hearts occurs through a posttranscriptional mechanism. These data suggest formation of additional ERAD protein complexes as a primary compensatory response to defective VCP activity and malfunctioning ERAD in the heart. These changes in expression of ERAD proteins occurred at 2 months of age (Fig. 3, C and D), before development of cardiomyopathy in DTgVCPK524A mice. DTgVCPK524A hearts also showed increased protein expression of the ER-resident prolyl isomerase cyclophilin B (CypB) (43) and protein disulfide isomerase (Fig. 3, C and D), both of which participate in targeting of substrates for ERAD (44–46). In contrast, expression of BiP and calnexin, ER chaperones that assist with folding of the bulk of secretory proteins in the ER (47), were unaltered (Fig. 3, C and D). Thus, impairment of VCP activity in the heart is associated with dysregulation of ERAD proteins and a build-up of ubiquitinated proteins.

To directly examine the effects of VCP inhibition on cardiomyocytes, we treated cultured rat neonatal cardiomyocytes with the VCP inhibitor DeBQ for 8 h, which resulted in a substantial accumulation of ubiquitinated proteins (Fig. 4, A and B). These data indicate that VCP activity is necessary for protein quality control and ubiquitin proteasome-mediated degradation and that acute inhibition of VCP disrupts cardiomyocyte proteostasis.
The UPR is activated in response to ER stress and results in inhibition of translation and induction of expression of ER chaperones downstream of the three ER-membrane UPR sensors, activating transcription factor 6α (ATF6α), inositol-requiring enzyme 1α (IRE1α), and protein kinase R–like ER kinase (PERK) (48). The UPR thus serves as a parallel pathway to simultaneously enhance the protein folding capacity and reduce the protein folding load within the ER, whereas ERAD functions to remove terminally misfolded proteins from the ER for degradation by the UPS. Here we observed that VCP mutant hearts had increased levels of PERK and robust induction of the activated nuclear form of ATF6α (Fig. 5, A and B), indicating that the ATF6α and PERK branches of the UPR, but not the IRE1α pathway, are engaged in VCP mutant hearts, likely as a protective mechanism to cope with defective ERAD.

**Disruption of VCP activity causes cardiomyocyte nuclear dysmorphology**

To gain insight into cellular defects caused by disruption of cardiomyocyte VCP activity, we evaluated the subcellular architecture in transgenic hearts by transmission EM. The results demonstrated normal cardiomyocyte ultrastructure in single transgenic tTA control and DTgVCP hearts (Fig. 6 A). Although cardiac ultrastructure was normal in all genotypes at 6 weeks of age (Fig. S3), by 5 months of age, which precedes the cardiomyopathy phenotype, hearts overexpressing the ATPase-deficient VCP mutant exhibit prominent defects in cardiomyocyte nuclear morphology, including abnormal nuclear shape, nuclear fragmentation, and increased invaginations in the nuclear membrane (Fig. 6 A), suggesting that VCP activity is required to maintain nuclear structural integrity. These marked defects in nuclear morphology in DTgVCP K524A hearts at 5 months of age were not accompanied by defects in myofilament or mitochondrial structure (Fig. 6 A). We also performed EM at 10 months of age, when DTgVCP K524A hearts are overtly diseased (Fig. 1), which revealed even more severe nuclear dysmorphology in DTgVCP K524A cardiomyocytes, including the presence of intranuclear vesicles, intranuclear membraneless regions of low electron density, and mitochondrial ultrastructural abnormalities, whereas aged tTA control
and DTgVCP hearts retained normal cardiomyocyte nuclear and subcellular ultrastructure (Fig. 6A).

To investigate molecular alterations that may underlie or coincide with the observed cardiomyocyte nuclear dysmorphology in DTgVCPK524A hearts (Fig. 6A), we performed immunoblotting for nuclear lamins and components of the nuclear envelope and linker of nucleoskeleton and cytoskeleton complex in cardiac lysates from transgenic mice at 2 and 6 months of age. The results indicate an increased abundance of nuclear lamins and specific components of the nuclear envelope in hearts overexpressing the ATPase-deficient VCP mutant at 6 months of age (Fig. 6, B and C) prior to development of cardiomyopathy but following up-regulation of ERAD complex proteins (Fig. 3, C and D). Indeed, VCPK524A hearts showed substantial up-regulation of lamin A/C as well as lamin B1, whereas the expression of nuclear lamins was unaltered by overexpression of WT VCP (Fig. 6, B and C). VCPK524A-overexpressing hearts also had increased protein levels of the INM protein SUN2 and the linker of nucleoskeleton and cytoskeleton complex protein nesprin-1 at 6 months of age (Fig. 6, B and C).
Lamin-associated polypeptide 2 (LAP2)/H9252, an INM protein (49, 50), was up-regulated in hearts overexpressing WT VCP or VCPK524A at 2 and 6 months of age (Fig. 6, B and C), suggesting that VCP forms a complex with LAP2/H9252 at the cardiomyocyte inner nuclear membrane. However, the expression of the structurally related INM protein MAN1 was unaltered by VCP overexpression (Fig. 6B). Indeed, VCP has been reported to function in a protein quality control pathway at the INM (25, 26), which may also be the case in cardiomyocytes. In conclusion, impairment VCP ATPase activity in the heart is sufficient to cause severe defects in the structure and molecular composition of cardiomyocyte nuclei.

**The VCP interactome implies a role in cardiac ribostasis**

In an effort to gain further mechanistic insight into the molecular functions of VCP in the heart, we performed proteomics to identify VCP-interacting proteins by immunoprecipitating endogenous VCP from mouse hearts and performing unbiased mass spectroscopy (MS)-based peptide analysis. Notably, we identified a profound enrichment of RBPs, ribosomal proteins, and ribonucleoproteins (RNPs) in the cardiac VCP interactome (Fig. 7A and Table S1). These include the known VCP interactor and RBP Ataxin-2 (51) and several “L” ribosomal proteins (Rpl proteins) that compose the large ribosomal subunit, ribosomal “S” proteins (Rps proteins) that compose the small ribosomal subunit, ribosome-binding protein 1 (Rrbp1), and Uba52, a fusion protein of ubiquitin with Rpl40 (52). Also identified were eukaryotic elongation factors and translation initiation factors, heterogeneous nuclear ribonucleoproteins (hnRNPs), DEAD-box RNA helicases (Ddx and Dhx proteins), and the nucleolar proteins nucleophosmin (NPM) and nucleolin (Fig. 7A). Importantly, many of these VCP-interacting proteins have been identified as components of RNP granules, higher-order cytoplasmic complexes of RNA and RBPs that accumulate in response to translation inhibition. These include...
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hnRNPs, staufens (Stau1 and Stau2), G3BP1/2, Ddx helicases, and euakaryotic initiation and elongation factors (53–57). Taken together, these data suggest that VCP has important functions in the regulation of ribosome remodeling and assembly and/or dissociation of complexes of ribosomal proteins, RNA, and RBPs, which could affect RNP granule dynamics and ribosome homeostasis.

As a complementary experimental approach to discover proteins that directly associate with VCP, we performed yeast two-hybrid (Y2H) screening using the N-terminal cofactor binding domain of VCP as bait (Fig. 7B). The known VCP cofactors Nsf11c (also known as p47) (17) and Ubxn2a (58) were sequenced several times (Fig. 5B), validating the Y2H approach. Importantly, the large ribosomal subunit protein Rpl12 and the translation initiation factor Eif3a were also sequenced several times (Fig. 7B), substantiating the association of VCP with ribosomal and ribosome-associated proteins in the heart (Fig. 7A). The small ribosomal subunit protein Rps20 and the known VCP cofactor Ufd1 (30) were also among the 41 unique clones identified by Y2H screening (data not shown). Thus, unbiased proteomics approaches collectively indicate predominant interactions of VCP with RBPs, ribosomal proteins, and ribosome-associated proteins.

It is also noteworthy that VCP was found to interact with the nucleolar proteins NPM and nucleolin in the heart (Fig. 7A), which was confirmed by immunoprecipitation of VCP and Western blotting (Fig. 7C). Moreover, NPM and nucleolin protein levels are up-regulated in VCPK524A hearts (Fig. 7D). VCP has been reported previously to function in the nucleolus (59), which serves as the preliminary site of ribosome biogenesis (33). Thus, VCP could potentially participate in processing or maturation of rRNA and preribosomes in cardiomyocyte nucleoli.

To investigate the functional consequence of the association of VCP with ribosomes and ribosome-associated proteins in cardiomyocytes, we processed hearts from transgenic mice into Triton-soluble and insoluble fractions and performed immunoblotting for ribosomal proteins. There was a substantial increase in the large ribosomal subunit proteins Rpl5 and Rpl7 in the insoluble fraction in hearts overexpressing ATPase-deficient VCP (Fig. 7E), suggesting that ribosomal proteins aggregate when cardiomyocyte VCP activity is inhibited. Immunohistochemistry further revealed accumulation and aggregation of the large ribosomal subunit proteins Rpl3 and Rpl19 in DTgVCPK524A cardiomyocytes (Fig. 7F), suggesting important cardiac functions for VCP in ribosome assembly, turnover, and/or remodeling. However, acute pharmacological inhibition of VCP activity in rat neonatal cardiomyocytes did not disrupt large ribosomal subunit protein localization (Fig. S4), suggesting that aggregation of ribosomal proteins caused by genetic inhibition of VCP activity in the heart may be secondary to abnormal ERAD activity and dysregulation of proteostasis. Indeed, genetic deletion of the ERAD component Sel1L in mouse pancreas also results in aggregation of ribosomal proteins (37), indicating that ERAD may functionally couple protein degradation and ER protein quality control with translation machinery to orchestrate synchronized regulation of proteostasis in vivo.

Discussion

Cardiomyocyte VCP activity is required for cardiac homeostasis and ERAD

Mutations in VCP cause multisystem proteinopathy 1 or IBMPFD, which affect skeletal muscle, brain, bone, and heart and account for roughly 50% of all familial multisystem proteinopathy disorders (60). Mutations in VCP are also thought to be responsible for 1–2% of familial ALS (61). The vast majority of the disease-causing mutations are missense mutations in the N-terminal cofactor and ubiquitin binding domain of VCP distal from its ATPase domains (62, 63); however, the underlying molecular etiology of these mutations and the mechanisms by which they cause disease are unclear. Therefore, to directly interrogate the functions of VCP in the heart, we mutated the Lys-524 residue within the D2 ATPase domain of VCP, which is known to be required for its activity (8).

Disruption of cardiomyocyte VCP ATPase activity in vivo by overexpression of an ATPase-deficient VCPK524A mutant resulted in age-dependent cardiomyopathy and an exacerbated cardiac disease response to pressure overload, underscoring the crucial functions of VCP in cardiac physiology and adaptive responsiveness. VCP is known to function in a myriad of ubiquitin-dependent cellular protein quality control pathways, including its well-described translocase functions in the ERAD pathway, where VCP extracts misfolded proteins from the ER for degradation by the UPS (7, 11–14, 16). Hearts overexpressing the VCPK524A mutant accumulated ubiquitinated proteins and up-regulated several ER membrane and ER luminal proteins that participate in the ERAD pathway, which is likely a compensatory response because of defective clearance of ERAD substrates in these cardiomyocytes. Interestingly, transgenic hearts overexpressing either WT or mutant VCP up-regulated protein expression of the ER membrane adaptor Sel1L, suggesting a stoichiometric complex between these two proteins. Expression of Sel1L is a critical determinant of the stability of the ERAD complex, including regulation of the requisite ERAD E3 ligase Hrd1 in the ER membrane (38). Thus, hearts overexpressing WT VCP likely enhance the stability and expression of a functional ERAD complex, whereas VCPK524A overexpressing hearts up-regulate the ERAD pathway, likely in a nonfunctional manner whereby misfolded substrates in the ER are fed into the ERAD complex and ubiquitinated but unable to be dislocated for degradation by the UPS. Indeed, similar to what we observed with inhibition of cardiac VCP activity, genetic knockdown of Hrd1 results in cardiac hypertrophy at baseline and augmented cardiomyopathy in response to pressure overload (36), providing further evidence of the critical role of ERAD in cardiac homeostasis and the response of the heart to pathological stress.

A previous study found a reduction in cardiac hypertrophy 2 weeks after TAC-induced pressure overload in cardiomyocyte-specific transgenic mice overexpressing WT VCP (64). Although we did not evaluate cardiac structure in our transgenic VCP mouse models until 4 and 8 weeks after TAC surgery, no reduction in cardiac hypertrophy was observed. Our WT VCP transgenic mice were generated on the same genetic background (FVB/N), and expression was also driven by the
α-MHC promoter, achieving similar robust overexpression as the previously reported transgenic model (64). Based on our results, it is likely that VCP overexpression in the heart only has minimal functional effects, given its already high abundance, accounting for upward of 1% of total cellular protein (65–67).

**VCP activity is necessary to maintain cardiomyocyte nuclear integrity**

Expression of the VCP<sup>K524A</sup> mutant in the mouse heart caused a dramatic alteration in nuclear morphology along with the appearance of intranuclear vesicles for the first time, as well as alterations in the molecular composition of the nuclear envelope and nuclear lamina. Although VCP has been reported to be involved in assembly of the nuclear envelope (23), we did not observe defects in cardiomyocyte nuclear morphology in DTgVCP<sup>K524A</sup> hearts until 5 months of age, suggesting that VCP does not directly mediate envelope assembly.

Interestingly, there was a stoichiometric up-regulation of the lamin-binding INM protein LAP2β with overexpression of either WT or mutant VCP at 2 months of age, suggesting that VCP forms a complex with LAP2β at the cardiomyocyte INM. Indeed, we observed VCP both in the cytoplasm and the nucleus in cardiomyocytes, as reported in other cell types (25, 26, 59, 68). The VCP homolog Cdc48 also functions in an INM quality control pathway of yeast (25, 26), and because the nuclear envelope is contiguous with the ER, it is possible that VCP functions in a pathway at the cardiomyocyte INM analogous to ERAD in the ER to regulate proteostasis of this entire linked compartment. Intranuclear aggregates are observed in cardiac and skeletal muscle myocytes from patients with IBMFD (3, 69), suggesting that the nuclear protein quality control functions of VCP may contribute to the molecular etiology of human disease. Indeed, the heart is particularly susceptible to disease caused by mutations in genes encoding proteins of the nuclear lamina or nuclear envelope (49, 70). Thus, cardiomyocyte nuclear defects likely contribute to cardiomyopathy in VCP<sup>K524A</sup> mice.

**VCP activity and cardiac ribostasis**

VCP can also function in ribosome-associated quality control to facilitate the release of polypeptides stalled in translation on ribosomes for degradation by the proteasome (27, 28, 30). We found that VCP directly interacts with ribosomal proteins, and impairment of cardiac VCP ATPase activity causes ribosomal protein aggregation, suggesting that VCP functions in ribosome biogenesis and/or remodeling in cardiomyocytes. VCP could participate in ribosome biogenesis within the cardiomyocyte nucleolus, particularly given its interaction with NPM and nucleolin, which could directly affect ribosome assembly and formation. Indeed, EM imaging of VCP<sup>K524A</sup> hearts at 10 months of age showed electron-opaque regions emanating from what appeared to be the nucleolus, as if these were regions with accumulating condensed ribonuclear proteins (Fig. 64). Indeed, we also observed aggregation of large ribosomal subunit proteins in the cardiomyocyte cytoplasm when VCP activity was inhibited, suggesting that VCP can function in ribosome processing, shuttling, turnover, or remodeling in the nucleus and cytoplasm. Unbiased proteomics identified associations of VCP with ribosomal proteins and translation factors, supporting a role of VCP in cardiomyocyte ribostasis.

It is possible that VCP<sup>K524A</sup> hearts exhibit aberrant ribosome quality control as a secondary response to stalled translation downstream of activation of the UPR. However, we observed specific and prominent effects on the ERAD pathway in VCP<sup>K524A</sup> hearts and believe that induction of PERK and activation of ATF6α occur as a secondary response to enhance the protein folding capacity of the ER to compensate for defective ER protein quality control. ER stress alone could not account for the direct interaction of VCP with ribosomes nor its previously established functions in translation stress (27), suggesting that VCP could have direct effects on ribostasis in the heart.

Our data indicate that VCP inhibition causes primary defects in ribosome quality control that take time to manifest in the adult heart to ultimately contribute to or even induce cardiomyopathy, as reminiscent of multisystem proteinopathy in the heart and other tissues caused by VCP mutations in human patients.

Overall, our results identify that VCP activity is critical for cardiac homeostasis and function. We found that VCP activity is necessary for proper cardiac ERAD and ribostasis as well as maintenance of cardiomyocyte nuclear structure. These pleiotropic phenotypes resulting from impaired VCP activity underscore the multifaceted molecular mechanisms whereby VCP mutations may cause disease in multiple organ systems in humans, especially in the heart.

**Experimental procedures**

**Animals**

Transgenic mice with cardiomyocyte-specific overexpression of WT VCP or VCP containing a point mutation of lysine 524 to alanine (K524A) were generated using the bigenic α-MHC promoter-driven transgene system (35), as described previously (71, 72). All mice were generated on the FVB/N genetic background. Briefly, a mouse VCP cDNA (Dharmacon, MMM1013-202859349) was subcloned into the SalI and Hin-dIII restriction sites of the α-MHC promoter-driven transgene system (35) and the resulting construct was digested with NotI to gel-purify the promoter−cDNA fragment for oocyte injection by the Transgenic Animal and Genome Editing Research Core at Cincinnati Children’s Hospital. To generate the VCP<sup>K524A</sup> mutant construct, mutagenesis was performed using the QuikChange II XL site-directed mutagenesis kit (Agilent Technologies) and the following primers: forward, 5′-GACCTCCT-GGCGTGTTGGGCAACCTTACTGCTAAG-3′; reverse, 5′-CTTTAGCGAGTGTTGCCCCACAGCCAGGAGGTC-3′. Transgenic mice in the FVB/N genetic background were bred and maintained on normal lab chow in the absence of tetracycline or doxycycline so that the bigenic α-MHC promoter-driven system remains fully induced at all times, which begins around birth in ventricular cardiomyocytes (35). Because no disease was observed in VCP transgenic mice, and disease in VCP<sup>K524A</sup> mice was not observed until after 6 months, we did not need to use the inducibility of this bigenic system. Echocardiography to evaluate cardiac function (72) and TAC to surgically induce pressure overload hypertrophy in 8-
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to 10-week-old mice (73) were performed as described previously. All animal procedures were approved by the Cincinnati Children’s Institutional Animal Care and Use Committee (protocol 2016-0069) and conformed to the NIH Guide for the Care and Use of Laboratory Animals. The number of mice used in this study reflects the minimum number of mice needed to achieve statistical significance (see “Statistical analysis”). The animals were not randomized and handled in a blinded manner, and both sexes were used. Analgesics were given to mice after the TAC surgical procedure to reduce pain (buprenorphine given subcutaneously at 0.1 mg/kg).

**Cultured neonatal rat cardiomyocytes**

Primary rat neonatal cardiomyocytes were cultured from disassociated hearts of 1- to 2-day-old neonatal rats exactly as described previously (72). Cardiomyocytes were cultured in M199 medium (Corning) with 1% BGS (Hyclone) for 48 h and then treated with DeBQ (Sigma-Aldrich) in M199 medium with 1% bovine growth serum for 8 h prior to harvesting for Western blotting.

**Western blotting**

Western blotting was performed as described previously (71). Proteins were extracted from mouse hearts or cultured cardiomyocytes in radioimmune precipitation assay buffer (50 mM Tris-HCl (pH 7.4), 1% Triton X-100, 1% sodium deoxycholate, 1 mM EDTA, and 0.1% SDS) with Halt protease inhibitors (Thermo Fisher Scientific) and sonicated with a Bioruptor UCD-200 (Diagenode), and lysates were cleared by centrifugation at 14,000 rpm for 10 min at 4 °C. Fractionation of mouse hearts into soluble and insoluble fractions was performed as described previously (74) with modifications. Hearts were homogenized in NET buffer (20 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 1 mM EDTA) with 1% Triton X-100 and protease inhibitors, sonicated, and clarified by centrifugation at 20,000 × g for 20 min at 4 °C, and then the supernatant was saved as the Triton-soluble fraction. The pellet was washed twice with PBS and protease inhibitors and then resuspended in urea buffer (7 M urea, 2 M thiourea, 4% CHAPS, and 30 mM Tris (pH 8.5)) with protease inhibitors, passed through an 21-gauge needle 10 times, sonicated, and clarified by centrifugation at 20,000 × g for 10 min at 4 °C to generate the insoluble fraction. Protein concentrations were determined using the DC Protein Assay (Bio-Rad) or the Pierce 660 nm Protein Assay (Thermo Fisher Scientific). Lysates were boiled in Laemmli buffer, resolved by SDS-PAGE, and transferred to PVDF membranes (Immobilon-FL, Millipore) for immunoblotting. Primary antibodies used were VCP (Abcam, ab36047, 1:500 or Novus Biologicals, NB120-11433, 1:1,000), Gapdh (Fitzgerald, 2433, 1:500), protein disulfide isomerase (Cell Signaling Technology, 2446S, 1:500), BiP (Sigma, G8918, 1:2000), α-tubulin (Sigma, T5168, 1:1,000), ATF6α-N (Signalway, SAB24383, 1:1,000), PERK (Cell Signaling Technology, 3192S, 1:1,000), IRE1α (Cell Signaling Technology, 3294S, 1:1,000), p-IRE1α (Abcam, ab48187, 1:1,000), Xbp1-s (Cell Signaling Technology, 83418S, 1:1,000), lamin A/C (Cell Signaling Technology, 2032, 1:500), lamin B1 (Cell Signaling Technology, 12586, 1:1,000), LAP2β (Bethyl Laboratories, A304-840A, 1:500), nesprin-1 (Abcam, ab192234, 1:500), nesprin-2 (Novus Biologicals, NB1P-84190, 1:500), SUN2 (Abcam, ab25914, 1:500), MAN1 (Santa Cruz Biotechnology, sc-50458, 1:500), Ran (Abcam, ab157213, 1:500), Gata4 (Santa Cruz Biotechnology, sc-1237, 1:250), Rpl7 (Abcam, ab72550, 1:500), Rpl5 (Abcam, ab86683, 1:500), hnRNPA1 (Abcam, ab50492, 1:500), α-sarcomeric actin (Sigma, A2172, 1:15,000), nucleophosmin (Abcam, ab10530, 1:500), and nucleolin (Abcam, ab22758, 1:1,000). Primary antibodies were followed by the appropriate fluorescent secondary antibodies (LI-COR Biosciences) and detection on an Odyssey CLx scanner (LI-COR Biosciences).

**Transmission EM and immunohistochemistry**

EM was performed on mouse cardiac tissue exactly as described previously (72). Hearts were perfusion-fixed in glutaraldehyde/cadaverine buffer, embedded in epoxy resin for sectioning, and then counterstained and imaged on a transmission electron microscope. Immunohistochemistry (IHC) was performed as described elsewhere (73, 75). Hearts were fixed in 4% paraformaldehyde for 4 h, incubated in 30% sucrose overnight, embedded in O.C.T (Tissue-Tek), frozen, and cryosectioned. Cryosections were fixed in methanol at −20 °C for 10 min, washed in PBS, blocked in IHC buffer (PBS, 5% goat serum, 1% BSA, 1% glycine, and 0.2% Triton X-100) for 1 h at room temperature, and then incubated with anti-Rpl3 (Proteintechnik, 66130-1-Ig) or anti-Rpl19 (Abnova, H0006143-M01) antibodies diluted 1:50 in IHC buffer overnight at 4 °C. Sections were then incubated with Alexa Fluor secondary antibodies (Thermo Fisher Scientific) diluted 1:1000 in IHC buffer for 1 h at room temperature and mounted with ProLong Gold Antifade Mountant (Thermo Fisher Scientific). Cultured neonatal rat cardiomyocytes were fixed in 4% paraformaldehyde and stained similarly using anti-Rpl6 (GeneTex, GTX114913, 1:50) or anti-Rpl7 (Abcam, ab72550, 1:100) antibodies. Staining with wheat germ agglutinin (Invitrogen) and quantification of cell surface area was performed in paraffin-embedded cardiac sections as described previously (72, 76). Image acquisition was performed on a Nikon A1 confocal microscope.

**Immunoprecipitation, Y2H, and proteomics**

Immunoprecipitation of mouse cardiac lysates was performed as described elsewhere (71). Mouse hearts were homogenized in lysis buffer (25 mM Tris-HCl (pH 7.4) and 5 mM EDTA) with protease inhibitors using a Dounce homogenizer. The lysate was spun down at 3,000 × g for 5 min at 4 °C, and the pellet was resuspended in NET buffer with 1% Triton X-100 and protease inhibitors, sonicated, and then immunoprecipitated with 5 μg of anti-VCP antibody (Novus Biologicals, NB1P120-11433) or IgG control (Santa Cruz Biotechnology) coupled to protein G Dynabeads (Thermo Fisher Scientific). Immunoprecipitates were washed three times in NET buffer with 1% Triton X-100 and boiled in Laemmli buffer for SDS-PAGE. Protein gels were transferred to PVDF membranes for Western blotting as described above or stained with the Prote-
oSilver Silver Stain Kit (Sigma) and submitted to the University of Cincinnati Proteomics Core Laboratory for MS sequencing by nano-LC-MS/MS and MALDI-TOF.

Yeast two-hybrid screening was performed as described previously [76] using the N-terminal domain (amino acids 1 to 208) of mouse VCP as bait and a mouse embryo cDNA prey library (Clontech). Plasmids purified from growing colonies were sequenced at the Cincinnati Children’s Hospital DNA Sequencing Core, and the identities of prey plasmids were determined using the Basic Local Alignment Search Tool (NCBI).

**Quantitative real-time PCR (qPCR)**

qPCR was performed exactly as described previously [76]. Briefly, RNA was isolated from mouse cardiac tissue using the RNEasy fibrous tissue mini kit (Qiagen). cDNA was synthesized using the Verso cDNA synthesis Kit (Thermo Scientific), and qPCR was performed with gene-specific primers and SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) on a CFX96 Real-Time PCR Detection System (Bio-Rad). The primer sequences for Sel1L [77], OS9 [37], and Herpud1 [78] are published elsewhere. The primer sequences for Gapdh were as follows: forward, 5’-TGCCCTCATGTGGTGTAGT-3’; reverse, 5’-TGTGGTACGAGCCCTTCC-3’. Expression was quantified using the standard curve method and normalized to Gapdh expression.

**Statistical analysis**

Data are represented as the mean ± S.D., and testing for statistical significance was performed using one-tailed Student’s t test or one-way analysis of variance (ANOVA) with post hoc Tukey’s test.

**References**

1. Custer, S. K., Neumann, M., Lu, H., Wright, A. C., and Taylor, J. P. (2010) Transgenic mice expressing mutant forms VCP/p97 recapitulate the full spectrum of IBMMD including degeneration in muscle, brain and bone. *Hum. Mol. Genet.* 19, 1741–1755 CrossRef Medline

2. Badanidi, M., Nalbandian, A., Watts, G. D., Vesa, J., Kitazawa, M., Su, H., Tanaya, J., Dec, E., Wallace, D. C., Mukherjee, J., Caiozzo, V., Warman, M., and Kimonis, V. E. (2010) VCP associated inclusion body myopathy and Paget disease of bone knock-in mouse model exhibits tissue pathology typical of human disease. *PLoS ONE* 5, e13183 CrossRef Medline

3. Hübbers, C. U., Clemen, C. S., Kesper, K., Bördrich, A., Hofmann, A., Kämäräinen, O., Tolksdorf, J., Stumpf, M., Reichielt, J., Roth, U., Krause, S., Watts, G., Kimonis, V., Wattjes, M. P., Reimann, J., et al. (2007) Pathological consequences of VCP mutations on human striated muscle. *Brain* 130, 381–393 CrossRef Medline

4. Watts, G. D., Wymer, J., Kovach, M. J., Mehta, S. G., Mumm, S., Darvish, D., Pestrakon, A., Whyte, M. P., and Kimonis, V. E. (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat. Genet.* 36, 377–381 CrossRef Medline

5. Meyer, H., Bug, M., and Bremer, S. (2012) Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system. *Nat. Cell Biol.* 14, 117–123 CrossRef Medline

6. Koppo, P., Espes, C. A., Förster, A., Zhang, X., and Freemont, P. S. (2012) Regulation of p97 in the ubiquitin-proteasome system by the UBX protein family. *Biochim. Biophys. Acta* 1825, 125–129 CrossRef Medline

7. Delabarre, B., Christianson, J. C., Kito, R. P., and Brungar, A. T. (2006) Central pore residues mediate the p97/VCP activity required for ERAD. *Mol. Cell* 22, 451–462 CrossRef Medline

8. Kobayashi, T., Tanaka, K., Inoue, K., and Kakizuka, A. (2002) Functional ATPase activity of p97/valosin-containing protein (VCP) is required for the quality control of endoplasmic reticulum in neuronally differentiated mammalian PC12 cells. *J. Biol. Chem.* 277, 47358–47365 CrossRef Medline

9. DeLabarre, B., and Brungar, A. T. (2003) Complete structure of p97/valosin-containing protein reveals communication between nucleotide domains. *Nat. Struct. Biol.* 10, 856–863 CrossRef Medline

10. Alexandru, G., Graumann, J., Smith, G. T., Kolawa, N. J., Fang, R., and Deshaies, R. J. (2008) UBXD7 binds multiple ubiquitin ligases and implicates p97 in Hif1α turnover. *Cell* 134, 804–816 CrossRef Medline

11. Rabino, N., Kerem, A., Fröhlich, K. U., Diamant, N., and Bar-Nun, S. (2002) AAA ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol. Cell* 22, 626–634 CrossRef Medline

12. Ye, Y., Meyer, H. H., and Rapoport, T. A. (2001) The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 414, 652–656 CrossRef Medline

13. Ye, Y., Shibata, Y., Kikkert, M., van Voorden, S., Wiertz, E., and Rapoport, T. A. (2005) Recruitment of the p97 ATPase and ubiquitin ligases to the site of retrotubulation at the endoplasmic reticulum membrane. *Proc. Natl. Acad. Sci. U.S.A.* 102, 14132–14138 CrossRef Medline

14. Greenblatt, E. I., Olzmann, J. A., and Kopito, R. R. (2011) Derlin-1 is a rhomboid pseudoprotease required for the dislocation of mutant e1-an-itrypsin from the endoplasmic reticulum. *Nat. Struct. Mol. Biol.* 18, 1147–1152 CrossRef Medline

15. Heo, J. M., Livnat-Levanon, N., Taylor, E. B., Jones, K. T., Dephoure, N., Ring, X., Jie, X., Brodsky, J. L., Madoe, F., Gygi, S. P., Ashrafi, K., Glickman, M. H., and Rutter, J. (2010) A stress-responsive system for mitochondrial protein degradation. *Mol. Cell* 40, 465–480 CrossRef Medline

16. Ye, Y., Meyer, H. H., and Rapoport, T. A. (2003) Function of the uFD1-Np4 complex in retrotubulation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. *J. Cell Biol.* 162, 71–84 CrossRef Medline

17. Kondo, H., Babouille, C., Newman, L., Levine, T. P., Pappin, D., Fremouw, P., and Warren, G. (1997) p97 is a cofactor for p97-mediated membrane fusion. *Nature* 388, 75–78 CrossRef Medline

18. Ramadan, K., Bruderer, R., Spiga, F. M., Popp, O., Baur, T., Gotta, M., and Meyer, H. H. (2007) Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin. *Nature* 450, 1258–1262 CrossRef Medline

19. Marie, M., Maculins, T., De Piccoli, G., and Labih, K. (2014) Cdc48 and a ubiquitin ligase drive disassembly of the CMG helicase at the end of DNA replication. *Science* 346, 125396 CrossRef Medline

20. Moreno, S. P., Bailey, R., Campion, N., Herron, S., and Gambus, A. (2014) Polyubiquitylation drives replisome disassembly at the termination of DNA replication. *Science* 346, 477–481 CrossRef Medline

21. Meerang, M., Ritz, D., Paliwal, S., Garajova, Z., Bosshard, M., Mailand, N., Janscak, P., Hübscher, U., Meyer, H., and Ramadan, K. (2011) The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks. *Nat. Cell Biol.* 13, 1376–1382 CrossRef Medline

22. Shih, Y. T., and Hsueh, Y. P. (2016) VCP and ATL1 regulate endoplasmic reticulum and protein synthesis for dendritic spine formation. *Nat. Commun.* 7, 11020 CrossRef Medline

23. Hetzer, M., Meyer, H. H., Walther, T. C., Bilbao-Cortes, D., Warren, G., and Mattaj, I. W. (2001) Distinct AAA-ATPase p97 complexes function in discrete steps of nuclear assembly. *Nat. Cell Biol.* 3, 1086–1091 CrossRef Medline
VCP activity regulates cardiac homeostasis

24. Zhou, H. L., Geng, C., Luo, G., and Lou, H. (2013) The p97-UBXD8 complex destabilizes mRNA by promoting release of ubiquitinated HtrU from mRNP. *Genes Dev.* 27, 1046–1058 *CrossRef Medline*

25. Foresti, O., Rodriguez-Vaello, V., Funaya, C., and Carvalho, P. (2014) Quality control of inner nuclear membrane proteins by the Asi complex. *Science* 346, 751–755 *CrossRef Medline*

26. Pantozioupolou, M., Boban, M., Foisner, R., and Ljungdahl, P. O. (2016) Cdc48 and Ubx1 participate in a pathway associated with the inner nuclear membrane that governs Asil degradation. *J. Cell Sci.* 129, 3770–3780 *CrossRef Medline*

27. Brandman, O., Stewart-Ornstein, J., Wong, D., Larson, A., Williams, C. C., Li, G. W., Zhou, S., King, D., Shen, P. S., Weizbezn, J., Dunn, J. G., Rouskin, S., Inada, T., Frost, A., and Weissman, J. S. (2012) A ribosome-bound quality control complex triggers deggregation of nascent proteins and signals translation stress. *Cell* 151, 1042–1054 *CrossRef Medline*

28. Defenouillère, Q., Yao, Y., Mouaiel, I., Namane, A., Galopier, A., Deconty, L., Doyen, S., Malabat, C., Saveau, C., Jaccquier, A., and Fromont-Racine, M. (2013) Cdc48-associated complex bound to 60S particles is required for the clearance of aberrant translation products. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5046–5051 *CrossRef Medline*

29. Defenouillère, Q., Zhang, E., Namane, A., Mouaiel, I., Jaccquier, A., and Fromont-Racine, M. (2016) RpC1 and Lm1 prevent C-terminal alanine-threonine tail (CAT-tail)-induced protein aggregation by efficient recruitment of Cdc48 on stalled 60S subunits. *J. Biol. Chem.* 291, 12245–12253 *CrossRef Medline*

30. Verma, R., Oania, R. S., Kolawa, N. J., and Deshaies, R. J. (2013) Cdc48/p97 activity regulates cardiac homeostasis, and survival. *Science* 346, 751–755 *CrossRef Medline*

31. An, H., and Harper, J. W. (2018) Systematic analysis of ribophagy in human cells reveals bystander flux during selective autophagy. *Nat. Cell Biol.* 20, 135–143 *CrossRef Medline*

32. Deisenroz, C., and Zhang, Y. (2010) Ribosome biogenesis surveillance: probing the ribosomal protein-Mdm2-p53 pathway. * Oncogene. 29*, 4253–4260 *CrossRef Medline*

33. Thomson, E., Ferreira-Cerca, S., and Hurt, E. (2013) Eukaryotic ribosome biogenesis at a glance. *J. Cell Sci.* 126, 4815–4821 *CrossRef Medline*

34. Lynch, J. M., Maillet, M., Vanhoultte, D., Schloemer, A., Sargent, M. A., Blair, N. S., Lynch, K. A., Okada, T., Aronow, B. J., Osinska, H., Prywes, R., Lorenz, J. N., Mori, K., Lawler, J., Robbins, J., and Molkentin, J. D. (2012) A thrombospondin-dependent pathway for a protective ER stress response. *Cell* 149, 1257–1268 *CrossRef Medline*

35. Sanbe, A., Gulick, J., Hanks, M. C., Liang, Q., Osinska, H., and Robbins, J. (2003) Reengineering inducible cardiac-specific transgenesis with an attenuated myosin heavy chain promoter. *Circ. Res.* 92, 609–616 *CrossRef Medline*

36. Doroudgar, S., Völkers, M., Thuerauf, D. J., Khan, M., Mohsin, S., Res-courty, L., Doyen, A., Malabat, C., Saveau, C., Jacquier, A., and Fromont-Racine, M. (2013) Cdc48-associated complex bound to 60S particles is required for the clearance of aberrant translation products. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5046–5051 *CrossRef Medline*

37. Leitman, J., Shenkman, M., Gofman, Y., Shtern, O. N., Ben-Tal, N., Hendershot, L. M., and Ledekremer, G. Z. (2014) Herp coordinates compartmentalization and recruitment of HRD1 and misfolded proteins for ERAD. *Mol. Biol. Cell* 25, 1050–1060 *CrossRef Medline*

38. Hosokawa, N., Kamiya, Y., Kamiya, D., Kato, K., and Nagata, K. (2009) Human OS-9, a lectin required for glycoprotein endoplasmic reticulum-association degradation, recognizes mannose-trimmed N-glycans. *J. Biol. Chem.* 284, 17061–17068 *CrossRef Medline*

39. Price, E. R., Zydowsky, D. L., Jin, M. J., Baker, C. H., McKeon, F. D., and Walsh, C. T. (1991) Human cyclophilin B: a second cyclophilin gene encodes a peptide-aryl proline isomerase with a signal sequence. *Proc. Natl. Acad. Sci. U.S.A.* 88, 1903–1907 *CrossRef Medline*

40. Bernasconi, R., Soldà, T., Galli, C., Pertel, T., Luban, J., and Molinari, M. (2010) Cyclosporine A-sensitive, cyclophilin B-dependent endoplasmic reticulum-associated degradation. *PLoS ONE* 5, e13008 *CrossRef Medline*

41. Grubb, S., Guo, L., Fisher, E. A., and Brodsky, J. I. (2012) Protein disulfide isomerases contribute differentially to the endoplasmic reticulum-associated degradation of apolipoprotein B and other substrates. *Mol. Biol. Cell* 23, 520–532 *CrossRef Medline*

42. Sakoh-Nakagowaga, M., Nishikawa, S., and Endo, T. (2009) Roles of protein-disulfide isomerase-mediated disulfide bond formation of yeast Mnl1p in endoplasmic reticulum-associated degradation. *J. Biol. Chem.* 284, 11815–11825 *CrossRef Medline*

43. Braakman, I., and Hebert, D. N. (2013) Protein folding in the endoplasmic reticulum. *Cold Spring Harb. Perspect. Biol.* 5, a013201 *CrossRef Medline*

44. Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P., and Ron, D. (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat. Cell Biol.* 2, 326–332 *CrossRef Medline*

45. Stroud, M. J., Banerjee, J., Veevers, J., and Chen, J. (2014) Linker of nucleo-skeleton and cytoskeleton complex proteins in cardiac structure, function, and disease. *Circ. Res.* 114, 538–548 *CrossRef Medline*

46. Chu, A., Rassadi, R., and Stochaj, U. (1998) Velcro in the nuclear envelope: LBR and LAPs. *FEBS Lett.* 441, 165–169 *CrossRef Medline*

47. Albrecht, M., Golata, M., Wühlner, U., and Lengauer, T. (2004) Structural and functional analysis of ataxin-2 and ataxin-3. *Eur. J. Biochem.* 271, 3155–3170 *CrossRef Medline*

48. Kobayashi, M., Oshima, S., Maeyashi, C., Nibe, Y., Otsubo, K., Matsu- zawa, Y., Nagaishi, T., Okamoto, R., Tsuchiya, K., Nakamura, T., and Watanabe, M. (2016) The ubiquitin hybrid gene UBAS2 regulates ubiquitination of ribosome and sustains embryonic development. *Sci. Rep.* 6, 36780 *CrossRef Medline*

49. Jain, S., Wheeler, J. R., Walters, R. W., Agrawal, A., Barsic, A., and Parker, R. (2016) ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell* 164, 487–498 *CrossRef Medline*

50. Plotter, D. S. W., and Parker, R. (2016) Principles and properties of stress granules. *Trends Cell Biol.* 26, 668–679 *CrossRef Medline*

51. Buchan, J. R., Kolaitis, R. M., Taylor, J. P., and Parker, R. (2013) Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. *Cell* 153, 1461–1474 *CrossRef Medline*

52. Kedersha, N., Panas, M. D., Achorn, C. A., Lyons, S., Tsisdale, S., Hickman, T., Thomas, M., Lieberman, J., Mclnerney, G. M., Ivanov, P., and Anderson, P. (2016) G3BP-Caprin1-USP10 complexes mediate stress granule condensation and associate with 40S subunits. *J. Cell Biol.* 212, 845–860 *CrossRef Medline*

53. Partridge, J. J., Lopreaiato, J. O., Jr, Latterich, M., and Indig, F. E. (2003) DNA damage modulates nucleolar interaction of the Werner protein with the AAA ATPase p97/VCP. *Mol. Biol. Cell* 14, 4221–4229 *CrossRef Medline*
VCP activity regulates cardiac homeostasis

60. Taylor, J. P. (2015) Multisystem proteinopathy: intersecting genetics in muscle, bone, and brain degeneration. *Neurology* **85**, 658–660. [CrossRef Medline]

61. Johnson, J. O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V. M., Trojanowski, J. Q., Gibbs, J. R., Brunetti, M., Gronka, S., Wu, J., Ding, J., McCluskey, L., Martinez-Lage, M., Falcone, D., Hernandez, D. G., et al. (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* **68**, 857–864. [CrossRef Medline]

62. Kimonis, V. E., Fulchiero, E., Vesa, J., and Watts, G. (2008) VCP disease associated with myopathy, Paget disease of bone and frontotemporal dementia: review of a unique disorder. *Biochim. Biophys. Acta* **1782**, 744–748. [CrossRef Medline]

63. Mehta, S. G., Khare, M., Ramani, R., Watts, G. D., Simon, M., Osann, K. E., Donkervoort, S., Dec, E., Nalbandian, A., Platt, J., Pasquali, M., Wang, A., Mozaffar, T., Smith, C. D., and Kimonis, V. E. (2013) Genotype-phenotype studies of VCP-associated inclusion body myopathy with Paget disease of bone and/or frontotemporal dementia. *Clin. Genet.* **83**, 422–431. [CrossRef Medline]

64. Zhou, N., Ma, B., Stoll, S., Hays, T. T., and Qiu, H. (2017) The valosin-containing protein (VCP) regulated by its N domain and C-terminal region. *Aging Cell* **16**, 1168–1179. [CrossRef Medline]

65. Vandermoere, F., El Yazidi-Belkoura, I., Slomianny, C., Demont, Y., Bidaux, G., Adriaenssens, E., Lemoine, J., and Hondermarck, H. (2006) The valosin-containing protein (VCP) is a target of Akt signaling required for cell survival. *J. Biol. Chem.* **281**, 14307–14313. [CrossRef Medline]

66. Song, C., Wang, Q., and Li, C. C. (2003) ATPase activity of p97-valosin-containing protein (VCP). D2 mediates the major enzyme activity, and D1 contributes to the heat-induced activity. *J. Biol. Chem.* **278**, 3648–3655. [CrossRef Medline]

67. Wang, Q., Song, C., and Li, C. C. (2004) Molecular perspectives on p97-VCP: progress in understanding its structure and diverse biological functions. *J. Struct. Biol.* **146**, 44–57. [CrossRef Medline]

68. Song, C., Wang, Q., Song, C., Lockett, S. J., Colburn, N. H., Li, C. C., Wang, J. M., and Rogers, T. J. (2015) Nucleocytoplasmic shuttling of valosin-containing protein (VCP/p97) regulated by its N domain and C-terminal region. *Biochim. Biophys. Acta* **1853**, 222–232. [CrossRef Medline]

69. Matsubara, S., Shimizu, T., Komori, T., Mori-Yoshimura, M., Minami, N., and Hayashi, Y. K. (2016) Nuclear inclusions mimicking poly(A)-binding protein nuclear 1 inclusions in a case of inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia with a novel mutation in the valosin-containing protein gene. *Neuromuscul. Disord.* **26**, 436–440. [CrossRef Medline]

70. Fatkin, D., MacRae, C., Sasaki, T., Wolff, M. R., Porcu, M., Frenneaux, M., Abtherton, J., Vidaileet, H. J., Jr., Spudich, S., De Girolami, U., Seidman, J. G., Seidman, C., Muntoni, F., Mühele, G., Johnson, W., and McDonough, B. (1999) Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N. Engl. J. Med.* **341**, 1715–1724. [CrossRef Medline]

71. Brody, M. J., Schips, T. G., Vanhoutte, D., Kanisicak, O., Karch, J., Maliken, B. D., Blair, N. S., Sargent, M. A., Prasad, V., and Molkentin, J. D. (2016) Dissection of thrombospondin-4 domains involved in intracellular adaptive ER Stress responsive signaling. *Mol. Cell Biol.* **36**, 2–12. [Medline]

72. Brody, M. J., Vanhoutte, D., Schips, T. G., Boyer, J. G., Bakshi, C. V., Sargent, M. A., York, A. J., and Molkentin, J. D. (2018) Defective flux of thrombospondin-4 through the secretory pathway impairs cardiomyocyte membrane stability and causes cardiomyopathy. *Mol. Cell Biol.* **38**, e00114-18. [Medline]

73. Brody, M. J., Feng, L., Grimes, A. C., Hacker, T. A., Olson, T. M., Kamp, T. J., Balijepalli, R. C., and Lee, Y. (2016) Lrrc10 is required to maintain cardiac function in response to pressure overload. *Am. J. Physiol. Heart. Circ. Physiol.* **310**, H269–H278. [CrossRef Medline]

74. Kim, H. J., Kim, N. C., Wang, Y. D., Scarborough, E. A., Moore, J., Diaz, Z., MacLea, K. S., Freibaum, B., Li, S., Molliex, A., Kanagaraj, A. P., Carter, R., Boylan, K. B., Wojtas, A. M., Rademakers, R., et al. (2013) Mutations in prion-like domains in hnRNPAB1 and hnRNPAl cause multisystem proteinopathy and ALS. *Nature* **495**, 467–473. [CrossRef Medline]

75. Brody, M. J., Cho, E., Myśliwiec, M. R., Kim, T. G., Carlson, C. D., Lee, K. H., and Lee, Y. (2013) Lrrc10 is a novel cardiac-specific target gene of Nkx2–5 and GATA4. *J. Mol. Cell Cardiol.* **62**, 237–246. [CrossRef Medline]

76. Brody, M. J., Hacker, T. A., Patel, J. R., Feng, L., Sadoshima, J., Tevosian, S. G., Balijepalli, R. C., Moss, R. L., and Lee, Y. (2012) Ablation of the cardiac-specific gene leucine-rich repeat containing 10 (lrrc10 results in dilated cardiomyopathy and conduction-system disease. *Circ. Physiol.* **310**, 237–246. [CrossRef Medline]

77. Quan, H., Fan, Q., Li, C., Wang, Y. Y., and Wang, L. (2018) The transcrip- tional profiles and functional implications of long non-coding RNAs in the unfolded protein response. *Sci. Rep.* **8**, 4981. [CrossRef Medline]

78. Paredes, F., Parra, V., Torrealba, N., Navarro-Marquez, M., Chiong, M., Bravo-Sagua, R., Troncoso, R., Pennanen, C., Karch, J., Cachero, C., Chiong, M., Caeser, C., Taylor, W. R., Molgo, J., San Martin, A., Jaimovich, E., and Lavandero, S. (2016) HERPUD1 protects against oxidative stress-induced apoptosis through down-regulation of the inositol 1,4,5-tris-phosphate receptor. *Free Radic. Biol. Med.* **90**, 206–218. [CrossRef Medline]