Desmin-related cardiomyopathy: an unfolding story

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Submitted 16 June 2011; accepted in final form 17 July 2011

McLendon PM, Robbins J. Desmin-related cardiomyopathy: an unfolding story. Am J Physiol Heart Circ Physiol 301: H1220–H1228, 2011. First published July 22, 2011; doi:10.1152/ajpheart.00601.2011.—The intermediate filament protein desmin is an integral component of the cardiomyocyte and serves to maintain the overall structure and cytoskeletal organization within striated muscle cells. Desmin-related myopathy can be caused by mutations in desmin or associated proteins, which leads to intracellular accumulation of misfolded protein and production of soluble pre-amyloid oligomers, which leads to weakened skeletal and cardiac muscle. In this review, we examine the cellular phenotypes in relevant animal models of desmin-related cardiomyopathy. These models display characteristic sarcoplasmic protein aggregates. Aberrant protein aggregation leads to mitochondrial dysfunction, abnormal metabolism, and altered cardiomyocyte structure. These deficits to cardiomyocyte function may stem from impaired cellular proteolytic mechanisms. The data obtained from these models allow a more complete picture of the pathology in desmin-related cardiomyopathy to be described. Moreover, these studies highlight the importance of desmin in maintaining cardiomyocyte structure and illustrate how disrupting this network can be deleterious to the heart. We emphasize the similarities observed between desmin-related cardiomyopathy and other protein conformational disorders and speculate that therapies to treat this disease may be broadly applicable to diverse protein aggregation-based disorders.

proteotoxicity; αB-crystallin; amyloid; heart

The cytoskeleton plays critical roles in cellular structure, cytoplasmic organization, proliferation, and intra- and intercellular signaling. The integrity and maintenance of the filament proteins that form the cytoskeleton is essential for proper functioning of mammalian cells. This is especially important in muscle cells, which rely on an intact and dynamic cytoskeleton to perform the functions of contraction and relaxation and connect major signaling hubs such as the sarcolemma and the nucleus to the contractile apparatus. Disruption of the cytoskeletal network can lead to a broad spectrum of myopathies that can present in the skeletal, cardiac, and/or smooth muscles. These diseases can lead to widespread muscle dysfunction and degeneration. They can be particularly serious when presenting in the heart, since unlike the majority of other striated muscles, the heart is never fully at rest and the cardiomyocytes are largely incapable of regeneration under normal physiological conditions.

One of the critical components of the cytoskeleton is the intermediate filament protein, desmin, which is a 53-kDa protein encoded on chromosome 2q35 in the human genome and is found in striated muscle (55). Desmin plays key structural and signaling roles in myocytes (15) and is critical for cytoskeletal organization and maintaining cardiomyocyte structure. Desmin filaments form an important part of the interconnected network that links the contractile apparatus to the rest of the myocyte: desmin filaments connect the sarcosome to the sarcolemma, extracellular matrix, and the nuclear lamina, as well as maintaining structural interactions at the Z-discs and intercalated discs, which link adjacent sarcomeres and myocytes, respectively (5, 15). Desmin filaments also interact with the mitochondria, ensuring their proximity to the A and I bands in the sarcomere so that the ATP produced is in close proximity to the energy-requiring structures. This ensures that the energy requirements of the contractile apparatus can be met. Thus the three-dimensional scaffolding of desmin maintains the architectural organization of the cytoskeleton and myofibrils, while influencing organelle positioning and connecting the functional regions of the myocyte. Additionally, desmin likely plays a role in mediating organelle trafficking, cell-cell communication, and signaling at the cardiomyocyte and myofibril levels (5).

Inherited and de novo mutations in desmin and accessory proteins can lead to desmin-related (cardio)myopathy (DRM) (9, 36). DRM is an example of a myofibrillar myopathy and can be caused by mutations in desmin, αB-crystallin (CryAB), myotilin, Z band alternatively spiced PDZ-motif protein (ZASP), Bcl-2-associated athanogene 3 (BAG-3), and filamin C (15). Regardless of the etiology of the disease and despite variance in clinical presentation, the phenotype at the cellular...
level is at least superficially very similar and is characterized by a disruption of the desmin filamentous network and an accumulation of large intracellular inclusion bodies that reside in the perinuclear region of cardiomyocytes. Although cytoplasmic inclusion bodies composed of intermediate filament-like fibers that positively stained for desmin had been previously described (11, 37), the first confirmed example of familial desminopathy was reported in 1994, in which a 52-yr-old man displayed muscle weakness and early onset cardiomyopathy. Subsequent analysis of the muscle tissue revealed large, granulofilamentous aggregates that positively stained with antibodies against desmin (14). Clinically, desminopathies present with widespread muscle weakness, particularly in the leg, facial, trunk, and respiratory muscles, and many patients end up confined to or largely dependent on a wheelchair for locomotion (15). Cardiac manifestations commonly include conduction blocks, which may necessitate pacemaker insertion. The loss of mitochondrial spatial organization is commonly observed (50). These cytoskeletal aberrations can lead to decreased contractility and Ca²⁺ handling, cardiomyopathy, and cardiomyocyte death (15, 23).

Similar to other protein conformation disorders, DRM is characterized by cytoplasmic aggregates of misfolded proteins that coalesce in a perinuclear location. In DRM, misfolded desmin appears to be the seed that facilitates aggregate formation; desmin knockout mice do not develop intracellular inclusion bodies (5, 15). Mouse models of DRM exhibit myofibrillar disarray, including disrupted sarcomere organization, loss of mitochondrial organization, and fibrosis (15, 30, 60). Cytoplasmic aggregates form in young mice and positively stain for CryAB, desmin, and ubiquitin. These cytoplasmic aggregates first appear as small, punctate dots distributed throughout the cytoplasm but, over time, are trafficked in a retrograde fashion along the microtubule network toward the perinuclear region (46).

While a cellular phenotype has been observed in clinical examples and animal models of mutations leading to DRM, the molecular mechanisms that lead to protein aggregation/inclusion body formation and eventually to cardiomyopathy remain elusive. This review focuses on recent advances characterizing the cell biology of DRM models caused by mutations to desmin and CryAB expressed the heart. Because specific phenotypic features of the human disease have been modeled in transgenic (TG) animals, these models have provided crucial details into the molecular mechanisms of cardiomyopathy caused by misfolded desmin, as well as insight into how desmin functions in the cardiomyocyte.

Intracellular Inclusion Body Formation

A characteristic feature of DRM is the formation of intracellular inclusion bodies composed of misfolded desmin and other proteins. Early work showed that inclusion bodies present in congenital myopathies positively stained for desmin (37) and that DRM was originally described in terms of the desmin-containing inclusions in skeletal and cardiac myopathies (13), whereas the etiology of disease remained obscure. With the use of genetic approaches, it was demonstrated that DRM could be caused by mutations in desmin (17, 35) or CryAB (54), which both led to the formation of aberrant, granulofilamentous aggregates of misfolded desmin in the cytosol (Fig. 1). The clinical phenotype suggests that aggregate formation in DRM is due to an accumulation of misfolded desmin; as noted above, mice that lack the protein, while still displaying a myopathic phenotype, do not contain the characteristic electron-dense protein accumulations (15, 35). CryAB, a member of the small heat shock protein family, is a chaperone for desmin, corroborating the likelihood that misfolded desmin is responsible for aggregate formation in DRM.

Several desmin mutations have now been linked to DRM (16, 18). A seven amino acid deletion in the desmin gene (DesD7), which removes residues encoding R173–E179 from the desmin gene, was identified in a 28-yr-old patient with a history of generalized muscle weakness (35). Muscle biopsies from this patient revealed ultrastructural abnormalities and aberrant distribution of desmin. Granulofilamentous, electron dense aggregates were observed in the sarcomeres. In vitro filament assembly experiments demonstrated the inability of DesD7 mutants to form native filaments; these data were suggestive that the loss of desmin functionality contributed to the disease phenotype (35). With the use of cardiomyocyte-specific mouse transgenesis, DesD7 was specifically expressed in the mouse heart to determine whether the mutation alone was sufficient to cause disease. Heterozygous DesD7 Tg mice displayed characteristic desmin aggregates in the cardiomyocytes’ perinuclear region, accompanied by general sarcomeric disorganization, hypertrophy, and progression to heart failure by 9 mo of age (58, 60). Similar phenotypic characteristics were observed in patients carrying desmin missense mutations, with cytoplasmic accumulations of abnormal, electron-dense aggregates (15). The expression of desmin missense mutations in cultured cells resulted in the lack of a coherent, filamentous network. In contrast to the expression of wild-type (WT) desmin, which led to increased filamentous networks, mutated desmin randomly accumulated in the cytoplasm (9). Taken together, the data suggest that mutant desmin is unable to form filaments and is sufficient to cause aggregate formation in DRM.

Supporting the hypothesis that misfolded desmin is the precursor to aggregates in DRM, it was observed that mutations in CryAB, a chaperone for desmin, also caused DRM and largely, but not exactly, phenocopied the protein aggregates observed in desmin mutations. The human Arg120Gly mutation in CryAB (CryABR120G) was first reported by Vicart et al. (54), who described a multigenerational French family diagnosed with DRM but with normal desmin. Muscle biopsies showed the characteristic cytoplasmic desmin aggregates, and genomic analysis identified the missense mutation in CryAB. Indeed, the expression of CryAB in C2.7 and BHK21 cells, which both contain desmin, resulted in cytoplasmic inclusion bodies positive for desmin and CryAB (Fig. 1). The inclusion body phenotype may be the result of the inability of desmin to attain or maintain its proper folded state because of a loss of CryAB chaperone function. Indeed, in vitro assays suggest that CryABR120G has reduced chaperone activity compared with the WT protein and can accelerate the aggregation kinetics of unfolded proteins (3). CryABR120G protein structure is substantially destabilized compared with normal protein, as evidenced by changes in circular dichroism spectra, reduced stability toward heat denaturation, and reduced protection of cells from heat shock (3, 6, 40). In vitro reconstitution with desmin revealed that CryABR120G induced aggregation of desmin in-
intermediate filaments (40). These alterations in chaperone activity likely stem from the structural changes to the /H9251-H9252-crystallin domain observed in CryABR120G, where perturbation to the curvature of the bottom /H9251-sheet leads to altered electrostatic interactions, which may affect chaperone function and oligomer dynamics (8). Thus the inclusion body formation may be the direct result of altered interactions between CryABR120G and desmin. This is supported by the lack of aggregate presence in CryAB knockout mice (4, 34), suggesting a toxic gain-of-function mechanism of CryABR120G in DRM.

More recent studies have attempted to more completely characterize the aggregates formed in DRM in vivo. Wang et al. (59) derived a TG mouse model expressing CryABR120G specifically in the heart, and this model recapitulated the disease phenotypes observed in humans, with the formation of perinuclear aggregates and progression to heart failure by 5–7 mo of age. The aggregates observed in this model ranged from large, regular, and low electron density to small, irregular aggregates with high electron density (59), which may indicate that aggregate formation is dynamic (Fig. 1). Progression to heart failure was worsened in TG mice expressing both CryABR120G and DesD7 (57), an expected result due to increased misfolded desmin. Likewise, overexpressing CryABWT attenuated the aggregation phenotype of DesD7 in cultured cells, likely because of an increased/accelerated removal of misfolded desmin before aggregation (57). These data show that CryABR120G is sufficient to cause DRM in murine models, and desmin aggregation can be mediated by mutant CryAB.

The large aggregates formed in CryABR120G appear to be morphologically similar or identical to aggresomes that have been described in numerous neurodegenerative disorders. Aggresomes are defined as insoluble cytoplasmic inclusion bodies that positively stain with Congo red and are trafficked in a retrograde manner along the microtubule network toward the perinuclear regions of cells, often coalescing at the microtubule organizing complex. Their formation is generally believed to result from deficits in protein degradation mechanisms (22). CryABR120G expression resulted in aggresome formation as initially defined by positive immunostaining of the visible aggregates for aggresomal proteins such as tubulin, secretory complex 61, and ubiquitin (46). The granulofilamentous aggregates were further defined as aggresomes based on experiments showing that their formation was blocked by the microtubule-depolymerizing agent colchicine (6). However, these experiments were done in cells that did not express desmin, so further study was needed to ascertain the desmin-dependent effects. Subsequently, similar results were observed in primary rat neonatal cardiomyocytes (RNCs); adenoviral expression of CryABR120G resulted in insoluble aggregate formation, whereas expression of CryABWT did not (46). Aggregates formed over time, with smaller “protoaggregates” forming early; these subsequently coalesced into large perinuclear aggregates, whose formation was completely ablated by treating the cells with nocodazole, a microtubule-depolymerizing agent. The aggregates positively stained for proteins associated with aggresomes characterized in other systems, including tubulin, other small heat shock proteins,

![Fig. 1. Desmin and αB-crystallin (CryAB) mutations cause desmin-related (cardio)myopathy (DRM). Immunofluorescent staining with anti-desmin (A and C) is detected by the green color. A and B: heart tissue from a nontransgenic (NTG) mouse. A: immunohistochemical staining for desmin. The intercalated disks are apparent. B: normal heart tissue contains regular sarcomeres (B) that exhibit a striated morphology with well-ordered mitochondria regularly positioned about the sarcomeres. C and D: mutations in desmin and CryAB disrupt the desmin cytoskeletal network, which perturbs normal organization of the myofibrils. These examples are of heart tissue from a DesD7 transgenic (TG) mouse. C: fluorescence micrograph showing sarcromere disruption and intracellular protein aggregates (white arrows) that positively stain for desmin. D: electron micrograph showing sarcomeric disarray, electron-dense aggregates (white arrows), and interrupted mitochondrial positioning about the sarcomere. The sarcomeric disorganization and aggregate formation are common phenotypes of DRM at the cellular level.](image-url)
and ubiquitin (46). Perhaps most striking was that CryABR120G-expressing cardiomyocytes, both in vitro and in vivo, positively stained with an antibody targeting the conformation of a pre-amyloid oligomer (PAO). This antibody recognizes the epitope of all types of soluble amyloid, regardless of sequence or etiology; it also recognizes oligomeric species of β-amyloid, α-synuclein, and human prion protein (21). This suggests that the structures of aggregates/PAO formed in CryABR120G have similar structures to the aggresomes and PAO found in neurodegenerative diseases (56). Indeed, CryABR120G aggregates positively stain with Congo red, an amyloid-binding dye, although they do not show the characteristic “apple green” birefringence under polarized light that is a hallmark of true amyloid (46). The processes involved in aggresomal formation and generation of PAO are diagrammed in Fig. 2.

Despite the aggregation phenotype observed in CryABR120G TG mice, it remains unclear whether the aggregates represent the toxic species in DRM. The observance of a soluble amyloid-like PAO species muddies the waters further, as PAO may represent the toxic species in many neurodegenerative diseases, with disease severity being better correlated to soluble PAO levels rather than plaque content (21). Thus it became critical to distinguish between and understand the toxicity profiles of the aggresomes compared with PAO.

The coexpression of normal CryAB (CryABWT) with CryABR120G was carried out using adenovirus-mediated expression in RNCs. The expression of CryABWT led to a significant decrease in aggresomal accumulation (47). However, the quantitation of PAO accumulation showed that the fraction of cytoplasm occupied by PAO more than doubled. Decreased aggresomal formation and enhanced PAO levels led to a significant increase in cytotoxicity. The apparent decrease in aggresomal accumulation, accompanied by decreased cell viability, implied that aggresome accumulation may be cytoprotective by effectively lowering levels of PAO (47).

The PAO hypothesis was subsequently tested using a cardiac-specific, inducible TG mouse model of CryABR120G, where the transgene can be turned on and off as desired (45, 47). These mice developed PAO and cytoplasmic aggregates and died of heart failure by 16 wk of age. Upon shutting off the transgene a few days before the first mice died, a reduction in PAO in the cardiomyocytes was observed, which corresponded to decreased pathologic hypertrophy and extended life span; however, the aggresome content was not reduced (47). To further test the hypothesis that PAO reduction can alleviate pathology, CryABR120G TG mice were placed on a voluntary exercise regimen, a treatment shown to reduce the amyloid levels and instill neuroprotection in mouse models of Alzheimer’s disease (24). Voluntary exercise reduced PAO formation.

Fig. 2. Genesis of aggresomes and pre-amyloid oligomers (PAO) in cardiomyocytes expressing mutant CryAB. Shown in schematic form are the processes that can lead to generation of the perinuclear aggregates or aggresomes and PAO in cardiomyocytes expressing CryABR120G. 1: Shown attached to a nascent sarcomere are polysomes (2) in the process of making sarcomeric proteins. Oligomers of mutant CryAB attach to the nascent polypeptide but are unable to mediate normal folding. 3a: Misfolded protein is recognized as such, ubiquitinated (ub-ub-ub) and targeted for degradation by the proteasome. The protein is degraded and the components recycled. 3b and 4: The misfolded protein, either because its size precludes it from entering the bore of the proteasome or because of compromised proteasome function, is not degraded and continues to form larger aggregates. 5: The aggregates are attached to dynein motors and are retrogradely transported down the microtubules to a perinuclear location. 6: These coalesce and form aggresomes, which are shown as electron dense, granulofilamentous aggregates near the nucleus (Ref. 58). N, nucleus; ag, aggresome. 7: It has been estimated that ~3,000 proteins have amyloidogenic potential. Under favorable circumstances, for those proteins, they can assume a parallel, β-sheet structure and form soluble PAO of indeterminate number (n). While the precise cause-and-effect relationships between the observed cellular phenotypes and corresponding cellular dysfunction are not clear, mitochondrial disruption, increased oxidative stress, and cardiomyocyte death can all result from the observed deficits in chaperone function, proteolytic activity, and PAO/aggregate generation.
in the hearts and decreased apoptotic markers, leading to an extended life span (28).

In a complementary approach, cardiac-specific transgenesis was used to express an expanded polyglutamine repeat (PQ83), a known PAO-genic peptide fragment (20, 21), to directly test whether the expression of PAO in cardiomyocytes led to cardiac disease. Indeed, mice expressing PQ83 in the heart displayed characteristic protein accumulations that positively stained with PAO-specific antibody (39). The mice developed pathological hypertrophy and progressed to heart failure, confirming PAO toxicity in the heart. The pathological consequences of PAO expression in the brain include disruption of cell membranes, inhibition of protein degradation pathways, and/or inactivation of functional proteins (32). In the heart, our laboratory demonstrated that the downregulation of nephrilysin, an enzyme involved in amyloid catabolism (28), was correlated with PAO accumulations, and there was also evidence of internal membrane disruption, with mitochondrial membranes being particularly susceptible in DRM-induced cardiomyopathy (39). However, the complete toxic spectrum of PAO accumulation in striated muscle has not yet been fully defined.

These studies underscore the similarities between CryABR120G-induced cardiac pathology and the neurodegenerative disorders brought about as a result of protein toxicity, suggesting that aggregate formation and toxicity mechanisms may be conserved between these ostensibly diverse diseases.

**Impaired Protein Degradation Mechanisms**

Protein aggregation and inclusion body formation can be the end result of impaired cellular proteolytic mechanisms (19, 64). Innate mechanisms exist to maintain the fidelity of cellular proteins, which ensures that proteins do not reach their proper folded conformation and are chaperoned such that they correctly fold or are targeted for degradation. The ubiquitin-proteasome system (UPS) and macroautophagy are the two primary mechanisms for removing misfolded proteins from the cell. Deficits in these degradation mechanisms can lead to an imbalance in protein homeostasis and increased steady-state levels of misfolded proteins that can aggregate (51). Improperly folded proteins often expose their hydrophobic core residues, which make them more prone to aggregate. Therefore, endogenous mechanisms for clearing the cytoplasm of unfolded protein intermediates are essential for maintaining the cell’s homeostatic balance and prevent the formation of toxic oligomers and aggregates.

The role of impaired mechanisms of protein quality control has been a significant focus in understanding the pathological mechanisms in DRM. While these protein degradation routes are used by all cells, protein quality control mechanisms are especially important in cells without overt proliferative capacity (e.g., cardiomyocytes), since a constant turnover of sarcomeric proteins is essential for maintaining cell viability and the activation of apoptosis to remove compromised cells could be detrimental to the tissue as a whole (63). The UPS is responsible for degrading most cellular proteins through a series of steps in which a protein is ubiquitinated and shuttled to the proteasome for degradation (51, 62, 64). It is becoming increasingly apparent that compromised proteasomal activity is an important contributing pathology to cardiovascular disease (48, 49) and that deficits in the UPS degradation pathway may play a role in the inclusion body phenotype of DRM (61). With the use of a fluorescent proteasomal substrate, green fluorescent protein ubiquitin receptor (GFPu), it has been shown that the proteasome can be inhibited by protein aggregation (2), an effect that has since been reproduced in cultured cardiomyocytes (10). In the CryABR120G mouse model, Chen et al. (7) showed that CryABG120G causes proteasomal impairment. When compared with non-TG (NTG) and CryABWT control mice, CryABG120G showed substantially higher levels of ubiquitinated proteins, whose appearance preceded aggregate formation. Crossing these mice with mice expressing a fluorescent reporter protein for proteasomal activity, green fluorescent protein ubiquitin degron (GFPdgn), resulted in abnormally high levels of the protein, which was no longer efficiently degraded by the proteasome. By 3 mo of age, the proteasomal reporter was observed in sarcoplasmic aggregates. Strikingly, despite an apparent impairment of proteasome function, specific activity assays for the trypsin, chymotrypsin, and caspase-like proteasomal activity showed increased activity in CryABR120G mice; these results highlight the limitations of those enzymatic assays in truly measuring proteasomal activity (61). A reduction in the 19S proteasomal subunit was observed over time, with a concomitant increase in 20S proteasome proteins, which is consistent with a decrease in substrate uptake into the proteasome (7). These effects can be recapitulated in other models of protein aggregation in the heart. In mutant desmin, DesD7 mice, increased amounts of ubiquitinated proteins were also observed, corresponding to decreased proteasomal degradation and increased proteasomal activity, with similar reductions in 19S proteins and increased 20S proteins observed in CryABR120G (26). These data suggest that defects in proteasomal degradation may also lie in the delivery of substrate to the proteasome, rather than the dysfunction of the proteasome itself. However, these studies do not address a crucial question: does the proteasome become inhibited by excess misfolded protein, or does the defective proteasome decrease degradation and lead to aggregation? Liu et al. (27) addressed this question by titrating the aggregation of DesD7 in RNCs and measuring proteasomal degradation as a function of aggregate levels (27). As AdDesD7 dosage was increased in cardiomyocytes expressing GFPu, GFPu content proportionally increased to DesD7 expression, suggesting that proteasomal degradation decreased as more misfolded desmin accumulated. When the chaperone proteins CryABWT and heat shock protein 70 (Hsp70) were expressed, the amount of misfolded desmin decreased, causing an increase in apparent proteasomal degradation as measured by GFPu levels. This effect was replicated using Congo red, a dye that inhibits amyloid aggregate accumulation in cardiomyocytes (46), with GFPu accumulation reduced when DesD7 cells were treated with the dye (27). These studies suggest that the amyloid-like aggregates formed in DRM can inhibit proteasomal degradation. Decreased proteasomal function over time may contribute to increased protein accumulation, deteriorating heart function, and progression to heart failure.

Macroautophagy, referred to as autophagy, has recently gained attention for its role in clearing misfolded protein species from the cytosol. Autophagic degradation involves the engulfment of cargo into double-membrane vesicles known as autophagosomes, which fuse with lysosomes to promote degradation of the cargo. Typical cargo degraded by autophagy...
includes misfolded proteins and defective organelles; autophagosomes can accommodate much larger cargo than the proteasome. Autophagy may therefore serve as the primary degradation route for large, oligomeric or aggregated protein species and cellular organelles such as mitochondria that have undergone cristolysis (44). Indeed, autophagy can be beneficial in decreasing morbidity in heart disease and other myopathies through the degradation of aggregate-prone proteins (33). As DRM is a bona fide cardiac proteinopathy (51), it was of interest to determine whether autophagy is involved in the pathogenesis. This question was addressed by Hill and colleagues, who observed increased autophagic vesicles in CryABR120G hearts using electron microscopy or measuring fluorescent GFP-LC3 positive puncta, compared with CryABWT-infected cells, suggesting an increase in autophagy as an adaptive response to misfolded protein generation (52). Inhibiting autophagy led to increased aggregate formation in CryABR120G-infected cells and drove more CryAB into the insoluble protein fraction relative to CryABWT-infected cells. To test the hypothesis that autophagy is increased in CryABR120G, CryABR120G TG mice were crossed with mice haploinsufficient for beclin-1, a crucial component of autophagosomal membranes; these mice should be deficient in autophagy (41, 52). Decreased beclin-1 caused increased aggregate formation in CryABR120G hearts, which was accompanied by decreased cardiac function and accelerated progression to heart failure in the absence of increased apoptosis. These data are consistent with the hypothesis that autophagy is upregulated in response to the production of misfolded proteins in CryABR120G hearts and may be an adaptive response to clear the cell of aggregate-prone species.

More recent studies investigated whether increasing autophagy can be protective in the CryABR120G model. Patterson et al. (38) hypothesized that overexpressing Atg7, a crucial component of autophagosome synthesis, would increase basal autophagic flux and lead to reduced protein accumulation in CryABR120G-infected RNCs. In fact, overexpressing Atg7 increased autophagic flux and led to a reduction in PAO and aggregate content in CryABR120G-infected RNCs. Conversely, a loss of function, mediated by small interfering RNA-mediated knockdown of Atg7, decreased autophagic flux, which increased PAO and aggregate content (38). Consistently, an autophagy-driven reduction of PAO/aggregate content strongly correlated with decreased cardiomyocyte death, whereas decreased autophagy increased cytotoxicity mediated by CryABR120G expression. Similarly, Zheng et al. (66) demonstrated increased autophagic flux in DRM mouse hearts, as well as an increase in the autophagy-related protein p62. The knockdown of p62 in RNCs expressing DesD7 or CryABR120G resulted in decreased autophagosome formation, leading to decreased cell viability (66). These data suggest that increasing autophagy could potentially be protective in DRM, and an adaptive autophagic response might compensate for the continued production of misfolded proteins. Interestingly, beclin-1 is downregulated in CryABR120G (31), a finding also observed in neurodegenerative disease (41). We speculate that, over time, autophagic degradative processes are compromised, leading to increased aggregation and cell death. The above data suggest that autophagy modulation may be a legitimate therapeutic avenue for the treatment of cardiac proteinopathies such as DRM.

Mitochondrial Dysfunction, Oxidative Stress, and Cell Death

DRM mutations result in early perturbations in mitochondrial structure and function (30), which may be related to the disease phenotype. Desmin is believed to contribute to mitochondrial function through maintaining proper mitochondrial positioning about the sarcomere (1). The disorganization of desmin can affect this positioning and, by extension, may compromise mitochondrial function, leading to cardiomyocyte death. Indeed, irregular mitochondrial shape and distribution (33), including aggregation of sarcolemmal mitochondria, occurs in desmin knockout mice, and this is correlated with weakened muscles and increased fatigue (25). Similarly, patients diagnosed with myofibrillar myopathies have reduced mitochondrial complex-I activities, and tissue biopsy samples show abnormal mitochondrial enzyme staining (43). Mitochondrial disorganization was observed in the DesD7 and CryABR120G mouse models of DRM (30, 58, 59). In NTG mouse hearts, mitochondria are well organized and in a regular pattern about the sarcomere (Fig. 3A). However, in mice expressing either DesD7 or CryABR120G, the mitochondrial spatial organization was highly perturbed when visualized by electron microscopy, and the myofibrils were interspersed with...
electron-dense aggregates. Mice expressing desmin\textsuperscript{WT} and CryAB\textsuperscript{WT} were identical to NTG controls, eliminating the possibility that mitochondrial disorganization was a result of TG protein overexpression. A study by Maloyan et al. (30) attempted to characterize the mitochondrial effects of CryAB\textsuperscript{R120G} expression in the heart. Although gross mitochondrial morphology was normal, significant disruption of mitochondrial organization was observed at 6 wk of age, before any overt, functional cardiac phenotype as determined by echocardiography (Fig. 3, B and C). Despite the normal morphology, mitochondrial oxygen consumption was dramatically reduced. This reduction was observed with glutamate/malate as substrate but was not seen with succinate or N,N',N'-tetramethyl-p-phenylenediamine (TMPD) + ascorbate, suggesting a deficit in complex-I activity. Mitochondrial permeability transition pore opening was also compromised in these hearts compared with NTG activity. Mitochondrial permeability transition pore opening to oxidative stress, and cell death. The metabolic abnormalities associated with CryAB\textsuperscript{R120G} pathology are intriguing, as noted in the above paragraph, mitochondrial complex-I function is diminished in CryAB\textsuperscript{R120G} hearts, which could be a result of increased oxidative stress. Indeed, in 4-mo-old CryAB\textsuperscript{R120G} mice, increased reactive oxygen species (ROS) production was detected relative to normal littermates (29). When oxypurinol, a xanthine oxidase inhibitor, was orally administered to the CryAB\textsuperscript{R120G} mice, ROS production and ROS-generating enzyme activity were substantially decreased. These effects led to a lessening of cardiac tissue fibrosis, a reduction in hypertrophic fetal gene program activation, and a compensatory up-regulation of glutathione reductase, suggesting that inhibiting ROS production lessened the pathology in CryAB\textsuperscript{R120G} TG mice. Furthermore, oxypurinol treatment largely rescued the defects in mitochondrial morphology and complex-I activity, although mitochondrial organization about the sarcomere remained perturbed. Despite the improvements in mitochondrial function, this treatment failed to rescue cardiac function. This suggests that while mitochondrial alterations certainly have a significant role in pathology, the myofibrillar stiffening that accompanies the accumulation of mutant CryAB (29) and/or the cytoskeletal defects that arise from a disrupted desmin filament network overcome the potential benefits of restored mitochondrial function.

Recent data on DRM have revealed many cellular phenotypes, providing visual and biochemical clues into the mechanisms of pathology. However, the mechanisms of cell death have not been well defined. As previously mentioned, it is disadvantageous for post-mitotic cells like cardiomyocytes to undergo apoptosis or other programmed cell death pathways, since the regeneration of this cell type is minimal. Nonetheless, evidence of apoptosis has been obtained in these models of DRM, with the mitochondria lysing and releasing proapoptotic molecules that trigger the activation of the intrinsic apoptotic pathway (30). Therefore, it is necessary to understand the role of apoptosis in the progression of the disease. To study this, CryAB\textsuperscript{R120G} TG mice were crossed with TG mice overexpressing Bel-2, a known inhibitor of apoptosis. As expected, these mice showed reduced markers of apoptosis, including decreased caspase activation and cytochrome-c release (31). These effects were accompanied by the conservation of normal mitochondrial morphology, reduced mitochondrial swelling, improved cardiac function, and a 20% increase in total life span. Autophagy also appeared to be increased, whereas aggregate accumulations were reduced by ~40%. However, increased necrotic cell death was noted, suggesting that the decrease in apoptosis resulted in an upregulation of alternative death pathways. The mechanisms of how the multiple cardiomyocyte death pathways talk to one another remain elusive, but it appears that decreasing apoptosis will not be an effective therapeutic avenue in DRM.

**Metabolic Abnormalities**

In DRM and for other disease models, it remains a significant challenge to translate mechanistic data gleaned from animal models into effective therapeutics for human disease. An attempt to create a humanized mouse model of CryAB\textsuperscript{R120G} was undertaken in which a human isoform of CryAB\textsuperscript{R120G} (hCryAB\textsuperscript{R120G}) was used to create a TG mouse with cardiomyocyte-restricted expression (42). Hsp25, another small heat shock protein that may act to compensate for the loss of chaperone activity from CryAB\textsuperscript{R120G}, was upregulated. Indeed, the increasing expression of other heat shock proteins or chaperones reduced aggregate formation in this model (6). Hsp25 downregulation has been correlated to reduced glutathione (GSH) production. Thus increased Hsp25 may increase GSH, which could elicit reductive stress in CryAB\textsuperscript{R120G} hearts. In agreement with this hypothesis, glutathione peroxidase and catalase, which increase hydrolysis of peroxides, were increased, corresponding to increased catalase protein levels. The concentration of reduced GSH and glutathione reductase (GSH-R) was significantly higher in hCryAB\textsuperscript{R120G} hearts than in NTG and CryAB\textsuperscript{WT} hearts, suggesting that the tissue is under reductive stress with accelerated antioxidative pathways. They speculated that increased GSH-R could be stimulated by increased glucose-6-phosphate dehydrogenase (G6PD), which can drive GSH-R production through increased oxidation of NADPH. G6PD expression was increased in the heart, and immunoprecipitation and colocalization experiments revealed that G6PD interacted with CryAB and Hsp25. Crossing these mice with mutant G6PD mice, which are deficient in functional G6PD, resulted in decreased CryAB aggregation in the heart and reduced hypertrophy. This effect on metabolism as a result of CryAB\textsuperscript{R120G} pathology is intriguing, but the interpretation is confounded by the use of an ectopic isoform of CryAB since in the TG model of CryAB\textsuperscript{R120G} expressing the mouse isoform, an oxidative stress phenotype is observed (29). At this point, these discrepancies can only be explained through the heterogeneity of the models used, and a definitive role for oxidation reduction in mediating CryAB\textsuperscript{R120G} pathology and potentially serving as a therapeutic avenue remains to be defined.

**Conclusions and Perspectives for the Future**

It is now clear that the desmin-related myopathies and cardiomyopathies are protein conformational disorders whose...
primary etiology lies in the improper folding of desmin. This primary lesion can lead to compromised mitochondrial function and structural weaknesses in the myocyte’s cytoskeletal networks, both of which can trigger multiple pathogenic sequelae. Post-mitotic cells appear to be particularly sensitive to protein conformation-based toxicity, possibly because if the aggregates cannot be cleared, misfolded species will continue to accumulate since the cell has limited or no potential to divide. If the misfolded or aggregated species cannot be cleared efficiently from the cardiomyocyte, perhaps critical concentrations of toxic species, particularly those having amyloidogenic potential, can accumulate. This can result in pathogenic concentrations of soluble amyloid-like PAA and eventually large aggresomes. At the cellular and biochemical level, the pathophysiology appears quite complex with defects to mitochondrial function, metabolism, redox processes, and cardiomyocyte biomechanics, all contributing to the overall pathology. As is true for the neurodegenerative diseases, the misfolded oligomers and/or inclusion bodies are likely involved in the pathology, and novel methods of increasing catabolism of these misfolded species before their accumulation may represent a viable therapeutic route. While the work presented herein provides detailed clues of disease pathogenesis, more data are needed to truly understand the progression of DRM and, more importantly, discover how to effectively treat it.

Many cardiomyopathies are caused by mutations in cardiac sarcomeric, cytoskeletal, and associated proteins. In general, it is becoming apparent that these mutations, as well as epigenetic stresses, can promote or cause protein misfolding and alter protein conformation. These changes subsequently impact on other proteins’ functions, interactions, trafficking, and turnover. Similarities between the physical manifestations of the DRM and neurodegenerative phenotypes are striking. Could the aggregation mechanisms between these seemingly diverse diseases be conserved? If so, deciphering the precise mechanisms of aggregate formation might uncover possible drug targets with wide applicability to protein aggregation-based disorders. For example, there is a single study showing that high doses of doxycycline, an antibiotic that inhibits amyloid formation (12), prolonged the life span of the CryABR120G mice (65). Future studies are needed to focus on identifying the cellular components that underlie and are responsible for pathogenic aggregation processes. We believe that this information will allow the field to move toward developing effective therapeutic strategies that can minimize tissue and organ damage resulting from protein misfolding and aberrant aggregation.

ACKNOWLEDGMENTS

We thank Hanna Osinska for providing the electron micrographs.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute (NHLBI) Grants P01-HL-69799, P50-HL-074728, P50-HL-077101, P01-HL-059408, and R01-HL-087862; a Fondation Leducq Award (to J. Robbins); and NHLBI Fellowship Award T32-HL-007382 to P. M. McLendon.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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