Viewing Extrinsic Proteotoxic Stress Through the Lens of Amyloid Cardiomyopathy

Proteotoxicity refers to toxic stress caused by misfolded proteins of extrinsic or intrinsic origin and plays an integral role in the pathogenesis of cardiovascular diseases. Herein, we provide an overview of the current understanding of mechanisms underlying proteotoxicity and its contribution in the pathogenesis of amyloid cardiomyopathy.

Macromolecule proteins comprise over 50% of a cell’s dry weight and are critical for virtually all cellular functions. Protein activity necessitates both the presence of the molecule and the proper three-dimensional conformation. In turn, imbalance between protein production and degradation or the presence of misfolded proteins result in a cellular proteotoxicity, marked by cellular dysfunction and/or death. Proteotoxicity has been implicated in myriad disease states, from neurodegenerative disease to heart disease to physiological aging, and is particularly pronounced in highly energetic cells with limited renewal, such as neurons and cardiomyocytes, that rely on protein homeostasis to maintain cellular function over the duration of an organism’s life span (51).

Such proteotoxicity may be initiated by either intrinsic or extrinsic sources. Intrinsic proteotoxic stress results from either excessive protein synthesis intracellularly or the production and aggregation of misfolded proteins that exceed the cell’s degradation capacity, and is typically localized to the cell wherein the protein is produced. In contrast, extrinsic proteotoxic stress arises from proteins produced by external sources and are aberrantly sensed or internalized, and exert toxic effects in the targeted cell. Although the downstream pathways driving and mitigating intrinsic proteotoxicity are better described, the molecular mediators that determine extrinsic proteotoxic stress are less clear.

Intrinsic Proteotoxicity

Intrinsic proteotoxicity is mitigated by both proteasomal and autophagic degradation pathways. Proteosomal degradation of misfolded protein can be initiated by signaling of the unfolded protein response (UPR) from the endoplasmic reticulum (ER) or via recognition by “quality control ligases” (12). The mode by which the cells signal these intrinsic responses has been described, and the role of the unfolded protein response in disease has been extensively reviewed elsewhere (39). Similarly, autophagic clearance of misfolded cytosolic aggregates or pre-amyloid oligomers is well described (51). Within the cardiovascular system, intrinsic proteotoxic stress plays an important role in the pathogenesis of many forms of heart disease. Expression and aggregation of intracellular polyglutamine repeat proteins can cause cardiac dysfunction in mice, inducing autophagy and, ultimately, necrotic cell death (35, 39). Similarly, germline mutations in humans in the structural protein desmin or chaperone protein α-crystallin (CryAB) result in aggregation of intrinsic misfolded proteins with pronounced cardiomyopathy, termed desminopathies. Overexpression of mutant CryAB in mouse models recapitulates intrinsic proteotoxicity with mitochondrial dysfunction, upregulation of various heat-shock proteins, increase in autophagic response, and ultimately cardiomyopathy (34, 37, 51). Intrinsic proteotoxicity may play an even more pronounced role in cardiac disease than has been appreciated. Recent work in idiopathic dilated cardiomyopathy has described fibrillar and oligomeric assemblies of misfolded proteins present in affected cardiomyocytes and their association with mutations in the presenilin gene, which is required for the processing of various cellular proteins (15, 42). Correspondingly, abnormal expression of presenilin results in cardiomyopathy in Drosophila models (22). The nature of these protein aggregates in patients with idiopathic dilated cardiomyopathy only recently has been described and was found to be composed of complexes of metabolic enzymes as well as coflin, a key regulator of the actin cytoskeleton (42). A mouse model of coflin haploinsufficiency recapitulated human phenotypes, with reduced ejection fraction and impaired cardiomyocyte contractility, suggesting a causal role for coflin in idiopathic dilated cardiomyopathy (42). While this literature continues to evolve and many mechanistic details remain to be uncovered, it has become increasingly clear that intrinsic proteotoxicity and related cellular processes are key contributing fac-
tors in heart disease (51). Less clear, however, have been the mechanisms, shared or unique, by which extrinsic proteotoxicity is mediated.

**Extrinsic Proteotoxicity**

Our current understanding of the mechanisms of extrinsic proteotoxic stress has been shaped in part by previous work in *C. elegans* on cell non-autonomous proteotoxicity, whereby secreted, misfolded proteins are able to exert proteotoxic stress on nearby or distant cells (30, 44). In higher organisms, extrinsic proteotoxicity can result when aberrantly folded proteins secreted by one cell exert toxicity on a second, localized cell, as is the case in Alzheimer’s disease, Parkinson’s disease, or amylin toxicity in pancreatic β-cells (23, 24, 52). Extrinsic proteotoxicity may also arise as protein secreted from one cell acts at multiple distant sites, with systemic manifestations. In either case, this extracellular protein undergoes conformational changes leading to the formation of β-pleated sheets and ultimately deposition in the extracellular space (41). First described by the famed pathologist Rudolph Virchow in the mid-19th century, these extracellular deposits were termed “amyloid” to reflect their waxy appearance and the grouping of diseases were termed amyloidoses (41). Early hypotheses describing the mechanisms of toxicity in amyloidogenic diseases had largely implicated passive infiltration of tissue with mature amyloid fibrils as the root cause of the disease pathology, particularly in the heart, whereby extracellular deposits impose passive restraint on proper filling and relaxation. However, current understanding also recognizes the role of toxic intermediates, and direct proteotoxicity resulting from their detrimental interaction with various cellular components, as critical to disease pathogenesis (4, 10, 40). In this review, we summarize the current understanding of extrinsic proteotoxicity within the heart, highlighting the study of systemic amyloidoses with cardiac manifestations as a window from which to view the fundamental mechanisms underlying extrinsic proteotoxicity.

**Amyloid Cardiomyopathy**

Amyloid cardiomyopathy is the manifestation of a number of extrinsic misfolded proteins, all of which are predisposed to deposition within the extracellular myocardium. Ultimately, these deposits induce dysfunction of the cardiomyocyte and coronary microvascular system. Among the proteins implicated in amyloid cardiomyopathy, monoclonal immunoglobulin light chain proteins (LC), transthyretin (TTR), and amylin proteins are the best characterized. Amyloid light chain (AL) amyloidosis is the result of plasma cell dyscrasia with systemic monoclonal immunoglobulin light-chain production and the potential formation of amyloid deposits in virtually all organ systems, including in kidney, liver, skin, peripheral nerve, and heart, with resulting organ dysfunction. Misfolding of both mutant and wild-type TTR protein in TTR amyloidosis has been implicated in peripheral neuropathy and cardiotoxicity, with certain mutations leading to an earlier age of onset (11). While not typically characterized as a systemic amyloidosis, hyper-amylinemia has been noted to cause peripheral vascular, cerebrovascular, and cardiac deposits of amyloidogenic amylin protein with resulting cardiomyopathy (5, 19). While the factors dictating specific organ tropism remain unknown, it is believed that features of both the precursor protein and mutated variant may potentially dictate localization. It remains unclear, however, whether there exist a unifying mechanism or even common components underlying all forms of amyloid cardiotoxicity. As the mechanisms underlying each of these seemingly related diseases begin to come into focus, so does the nature of extrinsic proteotoxicity.

**Mechanisms of Amyloidogenic Protein-Mediated Cardiotoxicity**

The primary mechanism underlying cardiac amyloidosis had long been believed to be the passive infiltration of extracellular space, which in turn mechanically disrupts tissue integrity and function. This notion, however, has been challenged by the observation that patients undergoing chemotherapy with removal of free circulating amyloidogenic proteins demonstrated improvement in cardiac function and symptoms without changes in tissue amyloid deposition (10, 38). Studies beginning in the early 2000s involving the administration of light-chain proteins isolated from patients revealed a role for soluble, circulating amyloidogenic light chains in direct cardiotoxicity that was specific for proteins isolated from patients with AL amyloidosis but not light-chain proteins isolated from patients with non-amyloidogenic multiple myeloma. Subsequent studies found only light-chain proteins from patients with cardiac involvement induced direct toxicity in isolated cardiomyocytes, with transforming growth factor-β-activated protein kinase 1-binding protein 1 (TAB1)-mediated p38 MAPK autophosphorylation occurring within minutes of exposure (28, 40). These early signals appear to propagate through downstream modulators, including stanniocalcin 1 (STC1), culminating at the levels of the nucleus and the mitochondria, resulting in decreased turnover of damaged mitochondria, increased reactive...
oxygen species (ROS) production, and decreased cellular ATP (4, 16, 17). Although it remains unclear whether these initial events are dictated by membrane receptor signaling and/or the interaction of internalized amyloidogenic proteins with cytosolic mediators, many groups have indicated that amyloidogenic light-chain proteins can be internalized by both cardiac fibroblasts and cardiomyocytes. Once internalized, light-chain proteins have been shown to localize to the perinuclear lysosomal compartment and to the mitochondrion (20, 21, 29). Most importantly, impaired lysosomal activity has been noted with exposure to amyloidogenic light-chain proteins, resulting in decreased autophagic clearance of damaged mitochondria and leading to increased production of ROS (16). The defective autophagy and lysosomal dysfunction may also impact accumulation of misfolded light-chain proteins in cardiomyocytes, which may potentiate the direct proteotoxicity of AL light-chain proteins in a feedforward loop. Additionally, amyloidogenic light-chain proteins even have been found to interact directly with mitochondrial proteins and enzymes, potentially impairing mitochondrial function through secondary effects on metabolism (20). Similarly, both mutant TTR and amylin amyloidoses induce extrinsic proteotoxicity in isolated cardiomyocytes, with mitochondrial injury and increased oxidative stress implicated as key mechanisms in the cellular response, suggesting a common final mechanism in the pathway of amyloid-related extrinsic proteotoxicity (1, 2). Interestingly, for mutant TTR, this proteotoxicity appears to be mediated by extracellular mechanisms, with amyloidogenic, proteotoxic V122I-TTR associated with extracellular proteins when exposed to cardiomyocytes, whereas non-amyloidogenic, non-proteotoxic T119M-TTR is readily internalized (25). Furthermore, whereas a membrane-bound receptor has been found to mediate amylin proteotoxicity, amylin also appears to be internalized and localizes to the sarclemma in both rodent and cardiomyocyte models of hyperamylinemia (1, 6). While a detailed understanding of mechanisms underlying amyloid cardiotoxicity still remains to be elucidated, the existing body of literature has indicated a potentially universal role at the level of mitochondrial dysfunction in extrinsic proteotoxicity.

**Emerging Diagnostics and Therapeutics**

Given the importance of proteotoxic stress to the pathogenesis of cardiovascular disease, targeting both intrinsic and extrinsic proteotoxicity will become critical for diagnostic and therapeutic purposes. This is particularly critical for AL amyloid cardiotoxicity, which remains marked by poor patient prognosis and for which there are few treatment options (33). Given the systemic involvement in many proteotoxic conditions and vague symptomatology, diagnosis is often delayed, thus a critical component of treatment of these proteotoxic disease states will be highly sensitive as will specific diagnostic markers that enable identification of disease at an early time point (33). Currently, diagnosis is often achieved through the combination of advanced cardiac imaging, circulating biomarkers, and histological examination of tissue biopsy (27, 33). Circulating diagnostic markers for amyloid cardiomyopathy have largely been limited to nonspecific markers of cardiac injury, including NH2-terminal natriuretic peptide type B (NT-proBNP) and cardiac troponin T, with specificity for amyloid cardiomyopathy relative to other more common forms of cardiomyopathy coming from threshold cutoffs since NT-proBNP cardiac troponin T levels are often greatly elevated in amyloid cardiomyopathy (31, 32). Additionally, these biomarkers also impart crucial prognostic information, with greater elevations in patients with amyloid cardiomyopathy associated with greater risk of cardiovascular death (8, 31). Other markers, such as soluble suppression of tumorigenicity 2 (sST2), may also add prognostic value for patients with AL amyloid cardiomyopathy (9). While such measures may have important prognostic potential in identifying patients at particularly high risk for adverse outcomes, more specific markers of amyloid cardiomyopathy are needed for early diagnosis. Such specific biomarkers will likely evolve along with our understanding of the mechanistic basis for extrinsic proteotoxicity and may potentially involve readouts of common components of proteotoxic disease, including impaired mitochondrial metabolism.

Similarly, future advances in treatment of cardiac proteotoxicity will ideally take advantage of insights gained from studying the disease mechanisms in the various cellular and model systems. Given the importance of direct cardiotoxic response to circulating amyloidogenic proteins, even independent of tissue deposition current therapeutic efforts have focused on reducing circulating levels of amyloidogenic proteins. These approaches include traditional chemotherapeutic agents paired with autologous stem cell transplantation, immunomodulators, and drugs that impair the unfolded protein response, which exert a more specific killing effect on the cell producing mutant protein (27, 33). Additional novel methods are being developed to reduce circulating light-chain proteins. siRNA against the mutant light-chain proteins can reduce amyloidogenic protein production by plasma-cell clones in vitro as well as reduce circulating levels in mice with plasmacy-
of internalization (45). Similarly, receptor-mediated endocytosis of nephrotoxic amyloid deposits directly, since such antibodies targeting these deposits have been proposed as a therapeutic option (50). This may offer an interesting therapeutic avenue as structural changes seen via echocardiography are associated with therapeutic response and survival (13). Furthermore, recent studies have shown that amyloid deposition may also contribute to metabolic dysfunction and increased production of reactive oxygen species in isolated cardiomyocytes treated with amyloid fibrils (26). However, since circulating free light-chain protein levels are correlated with disease severity and have been implicated in the progression in disease models, it remains to be seen whether therapies disrupting amyloid fibrils without a concomitant decrease in circulating light chain will be effective in patient (10, 38). Future therapies should similarly involve targeting the extrinsic proteotoxic response in cardiomyocytes, targeting either upstream the initial signaling cascade(s) or downstream at the level of the mitochondria. As our understanding of disease mechanisms and the fundamental basis for extrinsic proteotoxicity continue to evolve, so should emerging treatment strategies.

Summary and Perspective

The last several years have witnessed a great advance in our collective understanding of extrinsic proteotoxicity and its role in mediating amyloidogenic diseases within the heart. Whereas the larger concepts have been formulated, fine detailing is still required to complete the picture of this biological process. An area of much interest is to understand the initial mechanisms dictating external amyloidogenic proteotoxic response and the specific role of protein internalization vs. cell surface receptor activation. Three potential mechanisms have been proposed to mediate internalization of amyloidogenic proteins in various forms of amyloidosis: annular ring pore formation, bulk pinocytosis, or receptor mediated endocytosis. Amyloidogenic light chains have been demonstrated to form annular rings in vitro (29, 55). Other amyloidoses have been marked by pore-forming annular ring intermediates, but it is unclear whether pore formation is essential to internalization of misfolded monomers (7, 49). The receptor-mediated endocytosis of nephrototoxic amyloidogenic light-chain proteins into mesangial cells has been proposed as an alternative mechanism of internalization (45). Similarly, receptor-mediated endocytosis of misfolded, soluble species and amyloid-beta have been implicated in prion disease and Alzheimer’s disease, respectively (43, 52), whereas endocytosis of amylin in pancreatic β-cells is mediated by both receptor-associated and macropinocytic mechanisms (47). Interestingly, the receptor-mediated internalization of amyloid-beta has been shown to be under the regulation of p38 MAPK. p38 MAPK is activated upon binding of amyloid-beta to the nicotinic acetylcholine receptor α7nAChR, and the internalization of amyloid-beta is inhibited by incubation with p38 MAPK inhibitor SB202190 (52). Similar mechanisms may prove relevant to other amyloidogenic diseases, including AL amyloidosis, in which the toxicity of amyloidogenic light chains is mitigated by inhibition of p38 MAPK in isolated cardiomyocytes and in a zebrafish model of light-chain cardiotoxicity (28). As mentioned above, not all amyloidogenic proteins inflict a cardiotoxic response in similar ways, since notably mutant TTR appears to signal through non-endocytotic means (25). Given the potential importance of the initial sensing step in the downstream cellular toxic response, future work undoubtedly will focus on the nature of cell sensing of amyloidogenic proteins and on targeting these mechanisms as a therapeutic strategy.

Likewise, the common downstream pathways of cellular toxicity may be critical to disease pathogenesis and require further clarification. Intriguingly, in virtually all forms of proteotoxicity, both intrinsic and extrinsic, mitochondrial dysfunction and oxidative stress have been implicated as key components. This mitochondrial dysfunction may arise from signaling to the mitochondria from cytosolic components, through direct interaction of amyloidogenic proteins with mitochondrial proteins, and/or from impaired lysosomal clearance of damaged mitochondria, all of which have been implicated in proteotoxic response (16, 20). This common step in the progression of proteotoxicity may now allow for identification of specific metabolomic biomarkers of amyloid cardiotoxicity as well as potentially allow for new treatment strategies aimed at altering substrate utilization or bypassing enzymatic blocks in the mitochondria.

In addition to the sensing mechanisms and cellular responses to amyloidogenic light-chain proteins, the diversity of cell types impacted by this extrinsic proteotoxicity and the roles they play in pathogenic mechanisms remain unclear. In cardiac amyloid disease, the cardiomyocyte appears to be the predominant driver of disease pathology, although the contribution of other cells, such as endothelial cells or cardiac fibroblasts, to disease pathogenesis warrants investigation. Early evidence suggests cardiac fibroblasts may internalize amyloidogenic light-chain proteins with subsequent proteoglycan release that may impact dis-
ease phenotype (48). In amyloidogenic light chain-associated nephropathy, supporting mesangial cells are critical for processing amyloid proteins for fibril deposition in the extracellular space (46). Although the impact of this processing on disease pathogenesis remains unknown, supporting glial cells are also impacted by amyloidogenic proteins in neurodegenerative diseases (3, 36, 53). This concept of coupled proteotoxicity potentially opens new areas of biology and therapeutics for future discovery.

Amyloidogenic diseases serve as a model to understand the impact of extrinsic proteotoxicity on the heart. Early forays into this area of science and biomedicine have been quite revealing, altering our understanding of the pathobiology underlying amyloidoses and shedding important biological insight into proteotoxic mechanisms. Future work should continue to build on this foundation, with a goal of contributing to fundamental biology as well as uncovering new diagnostic and therapeutic strategies for diseases marked by extrinsic proteotoxicity.

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