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EDITORIAL COMMENT

Decoding the Nanoenvironment in Cardiac Amyloidosis Through Proteomics*

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By the help of microscopes, there is nothing so small, as to escape our inquiry; hence there is a new visible world discovered to the understanding.
—Robert Hooke, English scientist, 17th century (1)

Amyloidosis is a disease initiated by protein misfolding. These protein aggregates form amyloid fibrils, which are rigid, β sheet-rich deposits that have a conserved ultrastructure regardless of the precursor protein. Progressive amyloid deposition can occur in various tissues and ultimately lead to organ dysfunction. Cardiac amyloid diseases, in particular, are associated with significant morbidity and mortality because of heart failure, arrhythmia, and sudden death. Furthermore, cardiac amyloidosis confers a significant burden of heart failure, particularly among certain groups (i.e., African Americans, the elderly) (2). Transthyretin (ATTR) and light-chain (AL) amyloidosis are the most common forms of cardiac amyloidosis. In recent years, effective, targeted therapies have emerged for both AL and ATTR amyloidosis and have been shown to improve survival and morbidity due to organ damage (3–6). Anti-plasma cell chemotherapy can eliminate or significantly reduce the plasma cell clone that is producing amyloidogenic light chain. Transthyretin stabilizers and silencers can reduce tetramer breakdown and protein expression, respectively, to decrease the production of new fibrils (4–6).

Despite these substantial advances, important issues remain. First, most ATTR and AL therapies affect the production of new amyloid fibrils and have no or limited effect on amyloid already deposited in tissues (7). Unfortunately, disease awareness for cardiac amyloidosis remains limited, and many patients are still diagnosed with advanced-stage disease, when the efficacy of these novel therapies is attenuated (8). To date, the mechanisms of amyloid clearance and potential therapeutic targets are poorly defined.

Second, although the pathophysiology of ATTR and AL cardiac amyloidosis are both primarily characterized by progressive amyloid deposition, these diseases have very different prognoses. Untreated, the median survival for AL cardiac amyloidosis is 6 months, compared to 4 years for ATTR cardiac amyloidosis (9,10). This observation suggests that factors independent of fibril deposition can influence disease pathogenesis. Indeed, extensive preclinical studies have demonstrated that pre-amyloid species in AL amyloidosis can cause mitochondrial damage, impaired lysosomal autophagy, and endothelial dysfunction (11–13). Better understanding this fibril-independent, light chain cardiotoxicity will be crucial to further improve outcomes in AL amyloidosis.

Taken together, more information is needed about the biology of the amyloid plaque nanoenvironment, namely, the molecular pathways involved in amyloid pathogenesis and clearance from the myocardium. In this issue of *JACC: CardioOncology*, Kourelis et al. (14) provide intriguing novel insights into this

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understudied area. The authors examined cardiac tissue sections from a large biobank of patients with AL (n = 139) or ATTR amyloidosis (n = 292) or controls (e.g., individuals with nonamyloid cardiomyopathy or no known cardiac disease; n = 17). They extracted amyloid plaques or control myocardium using laser capture microdissection and then performed mass spectrometry to characterize the amyloid plaque proteomes. Key findings were as follows:

- The proteomic analyses identified 34 amyloid-specific proteins when compared to the control samples. Of these proteins, 13 were found in both AL and ATTR, 15 in ATTR only, and 6 in AL only.
- ATTR plaques had greater overall protein content and higher levels of myofilament and complement proteins, whereas AL plaques had more keratins.
- Among AL plaques, kappa AL was associated with higher levels of clusterin and a lower abundance of light chains.
- Higher amounts of PIK3C3, a phosphatidylinositol 3-kinase involved in autophagy, was associated with poorer survival in patients with ATTR.

This study has some limitations to note. First, the amyloid plaque proteomes were normalized and compared to the proteomes of the myocardium and interstitium in the control samples. The control patients tended to be younger than individuals in the amyloidosis groups. Second, the sample sources varied between the experimental and control groups (e.g., endomyocardial biopsy vs. autopsy; right ventricle vs. left ventricle). It is possible that these differences affected the proteomes for each group. Finally, the study identified unique AL and ATTR amyloid proteomes and demonstrated that PIK3C3 levels were associated with prognosis in ATTR. However, it is unclear whether there is a causal relationship between disease pathogenesis and the amyloid plaque proteins. Additional studies are needed to elucidate whether there is a mechanistic link between these proteins and cardiac amyloid disease progression.

Overall, the current study provides a timely, comprehensive atlas of the protein signatures in ATTR and AL cardiac amyloidosis. These data will support efforts to unravel the molecular pathways by which cardiac amyloid species promote tissue injury and evade the body's intrinsic mechanisms for protein clearance. The differences in the composition of ATTR and AL amyloid plaques are striking and warrant further investigations into how this may affect the contrasting prognoses of these diseases. Moreover, ATTR and AL proteomes should be compared to the known protein atlases of other amyloid diseases, such as Alzheimer disease, to find potential common pathogenetic pathways in amyloid diseases (15,16). Such discoveries could ultimately help address the current therapeutic gaps in cardiac amyloidosis.

AUTHOR DISCLOSURES

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