Current perspectives on cardiac amyloidosis

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Guan J, Mishra S, Falk RH, Liao R. Current perspectives on cardiac amyloidosis. Am J Physiol Heart Circ Physiol 302: H544–H552, 2012. First published November 4, 2011; doi:10.1152/ajpheart.00815.2011.—Amyloidosis represents a group of diseases in which proteins undergo misfolding to form insoluble fibrils with subsequent tissue deposition. While almost all deposited amyloid fibers share a common nonbranched morphology, the affected end organs, clinical presentation, treatment strategies, and prognosis vary greatly among this group of diseases and are largely dependent on the specific amyloid precursor protein. To date, at least 27 precursor proteins have been identified to result in either local tissue or systemic amyloidosis, with nine of them manifesting in cardiac deposition and resulting in a syndrome termed “cardiac amyloidosis” or “amyloid cardiomyopathy.” Although cardiac amyloidosis has been traditionally considered to be a rare disorder, as clinical appreciation and understanding continues to grow, so too has the prevalence, suggesting that this disease may be greatly underdiagnosed. The most common form of cardiac amyloidosis is associated with circulating amyloidogenic monoclonal immunoglobulin light chain proteins. Other major cardiac amyloidoses result from a misfolding of products of mutated or wild-type transthyretin protein. While the various cardiac amyloidoses share a common functional consequence, namely, an infiltrative cardiomyopathy with restrictive pathophysiology leading to progressive heart failure, the underlying pathophysiology and clinical syndrome varies with each precursor protein. Herein, we aim to provide an up-to-date overview of cardiac amyloidosis from nomenclature to molecular mechanisms and treatment options, with a particular focus on amyloidogenic immunoglobulin light chain protein cardiac amyloidosis.

heart; cardiomyocytes

Introduction

Amyloidosis represents a group of diseases that are characterized by extracellular deposition of amyloid fibrils in organs and tissues (61). These amyloid fibrils, as shown in Fig. 1, are composed of misfolded protein aggregates that arrange themselves in an antiparallel β-sheet form and manifest as insoluble rigid, nonbranching fibrils of 7.5–10 nm in diameter when viewed via electron microscopy (97). The term “amyloid” originated over 150 years ago as a descriptor for the “waxy” material observed in affected tissues, with the misconception that the deposits were from starch-like materials (amyl = “starch,” and -oid = “like”) (54). It was later realized, however, that amyloid fibrils are in fact composed of misfolded proteins that result from either excess production of or specific mutations in precursor proteins. To date, at least 27 distinct amyloid precursor proteins, each with heterogeneous protein sequence, structure, and function, have been identified to be amyloidogenic or capable of misfolding, aggregation, fibril formation, and tissue deposition (96). Abnormal amyloidogenic proteins may be either inherited or acquired through somatic mutations. Despite the diversity of precursor proteins, the final resulting nonbranched fibrils are largely indistinguishable from each other. Diagnosis of amyloidosis has largely centered on the identification of amyloid fibril deposition in pathology specimens using staining with Congo red, thioflavin T, or Alcian blue. With Congo red staining, amyloid fibrils exhibit a classic apple green birefringence when viewed using polarized light (30).

Amyloidosis may manifest as either a localized or systemic disease, depending on whether a single organ or multiple organs are affected. The individual amyloid diseases are classified based on their precursor protein, which translates to specific characteristics of the disease presentation and governs patient treatment plans and prognoses (25, 26, 53). Of the 27 identified precursor proteins which may result in human amyloidoses (Table 1), thus far nine proteins have been shown to potentially result in cardiac involvement. The nomenclature used in this review follows the guidelines set forth by The Nomenclature Committee of the International Society of Amyloidosis (96). Typically, the name starts with “A” for amyloid and is followed by an abbreviation of the precursor protein, e.g., amyloidogenic immunoglobulin light chain protein (AL),...
Cardiac amyloidosis has been traditionally viewed as a rare or orphan disease, particularly compared with acquired cardiovascular diseases such as coronary artery disease and hypertensive heart disease. Ongoing efforts have suggested, though, that the rarity of cardiac amyloidosis may be more a reflection of its underdiagnosis, rather than true incidence. This may be particularly true in the transthyretin (TTR) amyloidoses. In general, diagnosis of amyloid cardiomyopathy has proven to be quite challenging, requiring cardiac biopsy and pathological evaluation for a definitive histological diagnosis in many patients. Cardiac involvement is probably often misdiagnosed during the early stages of the disease, when ventricular wall thickening due to amyloid infiltration may be misdiagnosed as left ventricular hypertrophy due to hypertension. In patients with confirmed amyloid deposition in other organ systems, wall thickening on echocardiography, particularly if associated with low voltage on the electrocardiogram, is highly suggestive of cardiac involvement, and cardiac biopsy is rarely needed in such cases (28). In contrast, senile systemic amyloidosis, and many of the TTR mutations almost exclusively involve the heart, and endomyocardial biopsy are usually needed for a tissue diagnosis. As the treatment strategies for amyloidosis strategies are highly dependent on the type of amyloid precursor protein, precise typing of the amyloid is critical. In many cases, such as gene-positive amyloid with typical findings of ATTR or a young patient with multiorgan involvement and clear evidence of a plasma cell dyscrasia, the diagnosis is clear, but there are many cases in which clinical features are non-specific or confusing. In such cases the most accurate diagnostic technique appears to be molecular analysis of the amyloid fibrils from a biopsy, using mass spectrometry (105).

Cardiac amyloidosis can be classified as primary (AL), secondary (reactive, AA), hereditary, senile systemic, or isolated atrial amyloidosis (Table 2). This classification is based on the different amyloidogenic precursor proteins that are deposited, and correspondingly, the treatment strategies and prognoses are distinct for each of the different types of cardiac amyloidosis. In developed nations, primary amyloidosis (AL), senile systemic amyloidosis, and familial amyloidosis are most prevalent; the latter two are caused by amyloid fibrils from wild-type and variant TTR (ATTR), respectively. AA is frequently associated with chronic infection and inflammatory conditions and results from amyloid deposition of serum AA proteins. It rarely affects the heart, and when it does, the clinical manifestations tend to be milde. Isolated atrial amyloidosis results from deposition of atrial natriuretic peptide and is generally a late-onset condition, which is of little clinical significance other than being associated with an increased incidence of atrial fibrillation. Rare amyloidogenic precursor proteins may also result in cardiac amyloid deposition, including mutant apolipoprotein A1 (40, 41, 70), fibrinogen (100), and gelsolin (12, 60). Additionally, recent studies have demonstrated oligomeric assembly-derived intracellular and extracellular amyloid-like fibril deposition in hearts of patients with idiopathic dilated cardiomyopathy (IDCM). This abnormal protein aggregation was found to affect calcium handling and contributed to cardiac dysfunction (33). Although the fibrils found in these patients with IDCM exhibited positive Congo red staining, the nature of the precursor proteins responsible for these amyloid-like fibrils remains to be determined. Importantly, further investigation is required to establish the causal relationships between the precursor protein for primary amyloidosis, and amyloidogenic transthyretin (ATTR), the precursor protein for senile or familial amyloidosis. The most critical and fatal manifestation of systemic amyloidosis is associated with cardiac involvement, termed “cardiac amyloidosis” or “amyloid cardiomyopathy.” Cardiac amyloidosis, particularly AL cardiomyopathy, results in a rapid decline in cardiac function, with symptomatic heart failure and development of amyloid cardiomyopathy. AL cardiomyopathy is unique in that most standard heart failure therapies, such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and digoxin, have low efficacy in treating this disease, partly because they are all poorly tolerated. To date, treatment options are limited, resulting in an overall 1.3-yr median survival from diagnosis in AL cardiomyopathy, comparable with the most aggressive forms of cancers (26, 53). In this review, we provide an overview of the current knowledge base and potential molecular mechanisms underpinning these diseases, as well as review recent experimental therapeutic strategies for cardiac amyloidosis with a concentrated focus on AL amyloid cardiomyopathy.

Cardiac Amyloidosis

Cardiac amyloidosis is defined by the presence of extracellular amyloid deposition within the heart, with infiltration occurring in all anatomical distributions, including the atria, ventricles, and perivascular space (most commonly within small vessels) as well as within the valves and conduction system in some cases (28). Amyloid fibril infiltration leads to biventricular wall thickening with impaired relaxation, disruption of ventricular organization, and the loss of ventricular elasticity and often is categorized clinically as a restrictive cardiomyopathy (22).

Fig. 1. Amyloid fibril deposition in heart tissue affected by AL cardiomyopathy. Amyloid fibrils are seen in the upper part of the electron micrograph of heart tissue taken from a patient with AL cardiomyopathy (courtesy of Dr. Federica del Monte). The amyloid fibrils are nonbranched rigid fibrils of a 10 nm diameter. Collagen fibrils, with a much larger diameter are seen in the lower part of the image.
relationship among amyloid precursor proteins, oligomers, and/or amyloid fiber in the development of cardiac pathology in patients with IDCM. Senile systemic amyloidosis is most associated with aging, and as the general population continues to advance in median age, it is suspected that the incidence of cardiac amyloidosis will only continue to increase.

AL Cardiomyopathy

Primary or AL amyloidosis may be the most common systemic amyloidosis in the US and occurs equally in men and women, mostly in those over 50 years of age. While this disease is thought to be uncommon, the disease incidence is similar to other well-known diseases such as Hodgkin’s disease with ~2,000 to 2,500 new cases each year (25, 26, 95). The underlying etiology of AL amyloidosis is a plasma cell dyscrasia, in which clonal expansion of plasma cells results in overproduction and secretion of monoclonal immunoglobulin light chain protein. This form of plasma cell dyscrasia is similar to that which occurs in multiple myeloma, and as such, 10–15% of myeloma patients also develop AL amyloidosis.

Table 1. Summary of currently known amyloid precursor proteins and amyloidosis

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Precursor of Amyloid Fiber</th>
<th>Precursor Protein Production Site</th>
<th>Type</th>
<th>Distribution (Involved Organs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL (29)</td>
<td>Immunoglobulin light chain</td>
<td>Bone marrow</td>
<td>Acquired</td>
<td>Systemic (kidney, heart, nervous system, liver, spleen, soft tissue)</td>
</tr>
<tr>
<td>AH (24)</td>
<td>Immunoglobulin heavy chain</td>
<td>Bone marrow</td>
<td>Acquired</td>
<td>Systemic (kidney, heart, liver, spleen, soft tissue)</td>
</tr>
<tr>
<td>ATTR* (61)</td>
<td>Transthyretin</td>
<td>Liver</td>
<td>Hereditary</td>
<td>Systemic (nervous system, kidney, heart)</td>
</tr>
<tr>
<td>AB₂M (42)</td>
<td>β₂ microglobulin</td>
<td>Ubiquitous in all nucleated cells</td>
<td>Acquired</td>
<td>Systemic (hemodialysis-associated, mainly joints)</td>
</tr>
<tr>
<td>AA (83)</td>
<td>Serum amyloid A</td>
<td>Liver</td>
<td>Acquired</td>
<td>Systemic (liver, kidney, spleen, heart, gastrointestinal)</td>
</tr>
<tr>
<td>AApoAI (61)</td>
<td>Apolipoprotein AI</td>
<td>Liver and small intestine</td>
<td>Hereditary</td>
<td>Systemic (Liver, kidney)</td>
</tr>
<tr>
<td>AApoAI (61)</td>
<td>Apolipoprotein AI</td>
<td>Liver and small intestine</td>
<td>Hereditary</td>
<td>Systemic (kidney, heart)</td>
</tr>
<tr>
<td>AApoAIV (8)</td>
<td>Apolipoprotein AIV</td>
<td>Intestine</td>
<td>Hereditary</td>
<td>Systemic (heart)</td>
</tr>
<tr>
<td>AGel (56)</td>
<td>Gelsolin</td>
<td>Ubiquitous</td>
<td>Hereditary</td>
<td>Systemic (cornea, cranial nerve)</td>
</tr>
<tr>
<td>ALys (74)</td>
<td>Lysozyme</td>
<td>Ubiquitous</td>
<td>Hereditary</td>
<td>Systemic (kidney, liver, spleen)</td>
</tr>
<tr>
<td>AFib (99)</td>
<td>Fibrinogen chain</td>
<td>Liver</td>
<td>Hereditary</td>
<td>Systemic (kidney, sporadic heart)</td>
</tr>
<tr>
<td>ACys (73)</td>
<td>Cystatin C</td>
<td>Ubiquitous</td>
<td>Hereditary</td>
<td>Systemic (brain arteries and arterioles)</td>
</tr>
<tr>
<td>ABri/ADan (32)</td>
<td>ABriPP and ADanPP</td>
<td>Brain, kidney, pancreas</td>
<td>Hereditary</td>
<td>Localized (central neuron system)</td>
</tr>
<tr>
<td>ALect2 (5)</td>
<td>Leukocyte chemotactic factor 2</td>
<td>Liver</td>
<td>Acquired</td>
<td>Localized (kidney)</td>
</tr>
<tr>
<td>AβP (4)</td>
<td>Amyloid β protein precursor</td>
<td>Brain</td>
<td>Acquired</td>
<td>Localized (brain)</td>
</tr>
<tr>
<td>Aprp (31)</td>
<td>Prion</td>
<td>Infection agent</td>
<td>Acquired</td>
<td>Localized (brain, nervous system)</td>
</tr>
</tbody>
</table>

ACal (80) | Procalcitonin | Thyroid | Acquired | Localized (thyroid) |

AAIAPP (37, 43) | Islet amyloid polypeptide | Pancreas | Acquired | Localized (islets of Langerhans) |

AANF (48) | Atrial natriuretic factor | Atria | Acquired | Localized (atria) |

Apro (110) | Prolactin | Pituitary | Acquired | Localized (pituitary) |

Alas (111) | Insulin | Pancreas | Acquired | Localized (pancreas) |

AMed (39) | Lactadherin | Aortic media | Acquired | Localized (aortic media) |

Aker (64) | Kerato-epithelin | Eye | Hereditary | Localized (cornea) |

ALac (1) | Lactoferrin | Eye | Acquired | Localized (cornea) |

AGaap (65) | Odontogenic ameloblast-associated protein | Teeth | Acquired | Localized (teeth) |

ASEmL (58) | Semenogelin | Seminal vesicles | Acquired | Seminal (vesicles) |

*ATTR amyloidosis may represent 2 forms of amyloidosis: familial ATTR amyloidosis caused by mutant transthyretin protein, and acquired or senile ATTR amyloidosis caused by wild-type transthyretin protein. *Aβ amyloidosis may represent 2 forms of amyloidosis: familial Alzheimer’s disease and sporadic Alzheimer’s disease. *A1APP amyloid deposition was also found in kidney.

Table 2. Summary of common cardiac amyloidosisa

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Precursor of Amyloid Fiber</th>
<th>Type</th>
<th>Prevalence</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL (26)</td>
<td>Immunoglobulin light chain</td>
<td>Primary amyloid cardiomyopathy</td>
<td>50% in patients with AL amyloidosis</td>
<td>Median survival-11 mo</td>
</tr>
<tr>
<td>ATTR (28, 46, 79, 101)</td>
<td>Wild-type transthyretin</td>
<td>Familial amyloid cardiomyopathy</td>
<td>30% in patients with familial amyloidosis</td>
<td>Median survival-9–13 yr</td>
</tr>
<tr>
<td>AA (28)</td>
<td>Serum amyloid A</td>
<td>Senile systemic amyloid (SSA)</td>
<td>In most of patients with senile amyloidosis</td>
<td>Median survival-75 mo</td>
</tr>
<tr>
<td>AANF (48, 49)</td>
<td>Atrial natriuretic factor</td>
<td>Secondary amyloid cardiomyopathy</td>
<td>Rare, only 2% in patients with AA amyloidosis</td>
<td>Good</td>
</tr>
</tbody>
</table>

More than 40% in people older than 50

aOther cardiac amyloidosis include: AH (24), AApoAI (40, 41, 61, 69), AApoAIV (7, 8), AFib (99), and AGel (12, 60). However, these are rarely occurring with limited literature.
The precise mechanisms that regulate light chain protein misfolding and eventual fiber formation are unknown. It has been suggested that these light chain proteins often contain mutations which may facilitate protein misfolding, eventually leading to aggregation and assembly into amyloid fibrils (3, 52).

AL amyloidosis affects multiple organs in addition to the heart, including the kidney, liver, peripheral nervous system, and autonomic nervous system, with heart and kidney involvement most common, occurring in >50% of patients with AL amyloidosis. Cardiac involvement in AL amyloidosis patients is associated with the worst prognosis of any organ involvement (92) because of the development of a rapid AL cardiomyopathy, with overt heart failure and a median survival of <6 mo when untreated (25, 26). Pharmacological treatment strategies for AL cardiomyopathy include supportive care of cardiac function and elimination of the sources of light chain (87). Standard therapies for heart failure, including digoxin, calcium channel blockers, and β-blockers, are generally avoided because of their excess toxicity. Angiotensin-converting enzyme inhibitors and angiotensin-II receptor blockers are also poorly tolerated in patients with AL cardiomyopathy because of the potential for profound hypotension (26, 62). In AL cardiomyopathy, diuretics are often used to manage fluid status, though with great caution to avoid excessively lowering filling pressure and exacerbating insufficient cardiac output (62). Heart transplantation followed by chemotherapy and/or stem cell transplantation has been demonstrated to achieve a long-term remission and improve the survival in highly selected patients (17, 34). Heart transplant, however, is oftentimes not a viable option for patients with cardiac involvement due to the high incidence of multiorgan involvement and the rapid progression of disease in patients with AL amyloidosis (85). The mainstay therapeutic options for eliminating amyloidogenic light chain production was for many years the alkylating agents (specifically melphalan). High-dose melphalan with autologous hematopoietic stem cell transplantation was introduced two decades ago and has a good response rate but high morbidity and mortality in patients with severe cardiac amyloidosis, thereby needing careful patient selection (13).

Intriguingly, in patients with AL cardiomyopathy, the reduction of circulating free light chain levels following treatment is generally associated with a better clinical outcome despite a lack of obvious improvement in echocardiographic measurements (21, 88). Peptide biomarkers such as cardiac troponin and NH2-terminal pro-brain natriuretic peptide have been proposed for use as prognostic markers in patients with AL amyloidosis (20, 71). Based on serum levels of cardiac troponin and NH2-terminal pro-brain natriuretic peptide, a simplified staging system was developed to predict the median survival of AL amyloidosis patients (19). This convenient and highly reproducible system facilitates comparison across different studies to obtain information regarding the efficacy of different treatments. While a detailed elaboration of chemotherapy and stem cell transplantation are beyond the scope of this review, bortezomib, a proteasome inhibitor, which was initially developed for treatment of multiple myeloma, has clearly been proving useful. In fact, bortezomib may produce a more rapid response than melphalan. By inhibiting proteasome function, bortezomib triggers endoplasmic reticulum stress, resulting in plasma cell death. Recent studies in patients with AL amyloidosis have shown the efficacy of bortezomib, with or without dexamethasone, in eliciting a hematological response in 71% of patients in only 1.2 mo (median response time) (47, 82, 98). Another promising drug is thalidomide (and the similar agent lenalidomide). The combination of thalidomide with either melphalan or cyclophosphamide-dexamethasone, when administered to AL amyloidosis patients with cardiac involvement, showed increased tolerance and a high hematological response (109). While these reports are very encouraging, these chemotherapy agents are generally associated with neurotoxicity and (occasionally) worsening of heart or kidney function, among other significant side effects (90) and should be used under the supervision of a physician skilled in treating amyloidosis.

Despite the progress made over the past decades, the poor survival associated with AL cardiomyopathy poses a great need for collaborative efforts between clinicians and basic researchers to better understand the molecular mechanisms that promote disease pathogenesis and to develop advanced, targeted therapies.

**Mechanisms Underlying Tissue Damage**

The pathophysiology of AL cardiomyopathy was hypothesized to arise exclusively from fibril deposition in the extracellular space, with subsequent increased passive stiffness and a parallel loss of cardiac parenchyma. This potential mechanism of disease was further supported by physiological measures of primarily impaired filling and diastolic function in patients with amyloid cardiomyopathy (27, 51). Emerging evidence, however, has now challenged this notion and suggests that fibril deposition represents only one aspect of disease pathology, with light chain precursor proteins or aggregates themselves inducing direct cardiotoxicity, independent of fibril deposition. The first evidence for direct precursor protein toxicity originated with clinical observations that suggested a far worse outcome in patients with AL cardiomyopathy relative to ATTR cardiomyopathy, despite similar degrees of fibril deposition (23). Moreover, reduced circulating serum-free light chain following chemotherapy has correlated with improved cardiac function and prognosis in patients with AL cardiomyopathy, despite unchanged amyloid fibril infiltration in the heart (72). Because of the strong correlation between serum-free light chain levels and prognosis, an assay measuring free light chain levels was developed and is routinely used in managing AL amyloidosis patients, especially those with cardiac involvement. These clinical observations formed the basis for the hypothesis that light chain proteins themselves may be directly cardiotoxic.

The first experimental evidence to support the direct cardiotoxic effects of amyloidogenic proteins originated from work by Liao and colleagues (57), demonstrating that the infusion of amyloidogenic light chain proteins, purified from AL amyloidosis patients with severe cardiac involvement, but not light chain protein from myeloma patients without amyloidosis, resulted in diastolic dysfunction in an isolated mouse heart model. Further work demonstrated the direct cardiac toxicity of amyloidogenic light chain protein in cultured cardiac cells, with amyloidogenic light chain proteins increasing cellular oxidant stress, independent of fibril formation (11). The notion that amyloidogenic light chain proteins themselves may alter cardiac function has been subsequently confirmed by several researchers.
additional laboratories (63, 64, 94). Migrino and colleagues (63) have found higher levels of serum oxidative stress markers in patients with AL amyloidosis compared with healthy controls. They also found that human arterioles exposed to amyloidogenic light chain proteins results in the generation of reactive oxygen species and impairment of vascular smooth muscle cell relaxation. This was extended in a recent study where Migrino and collaborators (64) reported endothelial dysfunction following amyloidogenic light chain treatment coupled with increased apoptotic cell death of coronary artery endothelial cells. In addition to showing that isolated amyloidogenic light chain protein from patients conferred cardiac toxicity, Sikkink and Ramirez-Alvarado (94) were able to recapitulate cardiac toxicity by using recombinant light chain proteins with sequence specific mutations.

Insight into the mechanism by which amyloidogenic light chain causes toxicity and cellular dysfunction was highlighted in the studies reported by Shi et al. (93). Using both pharmacological inhibition of p38 MAPK and dominant-negative p38α MAPK adenovirus, Shi et al. reported that noncanonical p38 MAPK pathway activation plays a pivotal role in mediating amyloidogenic light chain-induced contractile dysfunction and apoptotic cell death in cultured cardiac cells. These observations were confirmed in vivo in mice. The infusion of amyloidogenic light chains by osmotic minipump increased apoptosis in cardiac tissue, and this was abolished in p38α MAPK dominant-negative mice, suggesting that p38α activation is essential for amyloidogenic light chain protein to execute its cardiac toxicity.

Taken together, the direct cardiac toxicity of amyloidogenic light chain protein has been shown to likely be an important factor in the pathogenesis of AL amyloidosis cardiomyopathy. These experimental data provide a strong notion for the elimination of circulating light chain proteins, either directly or through the removal of source plasma cells. Furthermore, molecular targets such as the p38 MAPK signaling axis offer additional potential targets for future therapeutic interventions to combat this dreadful disease.

**Mechanism of Amyloid Fibrillogenesis**

What determines the amyloidogenic properties of precursor proteins remains largely unclear. It has been shown that a number of proteins are prone to misfold and form amyloid fibrils with a predominantly antiparallel β-sheet secondary structure. This dynamic equilibrium between a native form and a misfolded form can be influenced by several independent factors, including, but not limited to, specific genetic mutations, abnormally high concentration of protein levels, destabilization of protein structure, dysfunctional protein degradation, or defective quality control machinery because of aging or other pathological conditions (61). In addition, a recent identification of intrinsic disordered protein (IDP) has extended the understanding of protein misfolding involved in amyloidosis (104). In contrast to conventional proteins, IDPs are naturally unfolded proteins that remain functional without having an ordered secondary or tertiary structure and transition to different folding states based on signaling or posttranslational modifications (103). For instance, the monomer of Aβ42, the precursor protein for Alzheimer’s disease, remains in unstructured IDP states (without either α-helix or β-sheet structure) and the assembly of Aβ42 fibrils starts with partially refolding to premolten globule-like conformation (50).

While significant efforts have been put forth to understand how precursor proteins undergo a series of conformation changes to misfold and eventually form amyloid fibril, the mechanisms guiding this process, particularly for AL amyloidosis, still remain poorly understood. Researchers have found that amyloidogenic light chain proteins are less stable compared with normal counterparts, which may contribute to protein misfolding (52, 68, 106, 107). That said, amyloidogenic light chain proteins have been found to exhibit a number of mutations without a clear genotype-phenotype association. Recent efforts have focused on those factors that may contribute to decreased thermostability of amyloidogenic light chain protein, including particular amino acid mutations, posttranslational modifications, and extracellular matrix environment (3, 15, 35, 52, 91, 101). Toward this, several reports have demonstrated that a single amino acid mutation present in an amyloidogenic light chain protein may result in significantly less thermostability and enhanced fibril formation (3, 59, 68, 77, 108). While this work on a relatively small number of light chain proteins is certainly encouraging, because of the heterogeneity of this disease, a larger sample size is needed to draw a definitive conclusion. Moreover, by comparing mutated amyloidogenic light chain proteins with normal light chain proteins, ongoing work has sought to determine mutational “hot spots” and protein sites that may alter posttranslational modification, including N-glycosylation (101). When comparing light chain sequences of 50-k and 91-λ light chain proteins from AL patients, Poshusta et al. (79) identified a significant difference in the number of nonconservative mutation between normal and AL light chain sequences in specific secondary structure elements. Moreover, the distribution of nonconservative mutations was correlated with serum-free light chain levels in AL patients. This may suggest a potential association between nonconservative mutations and amyloidogenesis in AL amyloidosis. The detailed molecular mechanism of how these nonconservative mutations contribute to misfolding and fibrillogenesis still remain to be explored.

In addition to the genetic mutations, several important posttranslational modifications have also been described in amyloidogenic light chain proteins. These posttranslational modifications include disulfide-linked dimerization, S-cysteinylation, glycosylation, fragmentation, S-sulfonation, and 3-chlorotyrosine formation (15). Dimerization has been suggested to be important to maintain the normal structure and stability (2). Davern et al. (16) chemically modified lysine residues of amyloidogenic light chain proteins with sulfo-NHS-biotin, which greatly enhanced binding to cells as well as altered conformational state, suggesting that posttranslational modifications of light chain may contribute to the amyloidogenesis and pathogenesis of the disease. Additionally, glycosaminoglycans (GAGs) have been found to be involved in amyloid fibril deposition, based on mass spectrometry analysis; however, the role of GAGs in the formation of amyloid fibrils remains unclear (67). Recent work from Ren et al. (83) has shown that the presence of GAGs promotes the formation of oligomers and fibrils of amyloidogenic light chain proteins but not non-amyloid myeloma light chain proteins. This highlights the interaction of light chain protein and extracellular matrix environment in the formation of amyloid fibril deposition. Moreover, serum matrix metalloproteinase-9
and tissue inhibitors of matrix metalloproteinase-1 have been suggested to be higher in patients with AL cardiomyopathy compared with patients with ATTR cardiac amyloidosis, suggesting that extracellular matrix proteolysis may play a role in this disease (9). Recent studies have shown that the extracellular chaperone molecule clusterin binds and stabilizes nonnative conformation of proteins to prevent their aggregation (69). Moreover, clusterin has been shown to protect the brain from the development of Alzheimer’s disease (69). A recent study has shown that clusterin is preferentially downregulated in the serum from patients with either ATTR or AL amyloidosis, suggesting that the loss of this extracellular chaperone may be associated with fibril deposition and disease pathogenesis (38). While the role of clusterin has not been investigated in the pathogenesis of AL cardiomyopathy, it remains an interesting target for future investigation.

Experimental and Evolving Therapies

The standard and newer therapies for AL cardiomyopathy described above currently include alkylating chemotherapy and high-dose melphalan with autologous hematopoietic stem cell transplantation (73). These treatments, however, are not viable options of all AL patients and are sometimes poorly tolerated (25, 26, 62). Therefore, it is of great importance to translate the basic science-driven understanding of amyloidosis into clinical drug development. The success of target therapy has been witnessed in the management of ATTR cardiomyopathy, in which stabilizing mutant and wild-type TTR with small molecules has proven to be effective in animal models and is currently being evaluated in clinical trials (NCT-00925002, NCT-01171859) (89). Several experimental therapeutic approaches that aim to target amyloidogenic light chain protein or amyloid fibrils resulting from AL amyloidosis are currently at various stages of development and are described below.

Small RNA interference (RNAi) technology has been used in an attempt to eliminate light chain production and has been found to have high specificity and low toxicity in experimental models (18, 86). Phipps and colleagues (78) used RNAi in an SP2/O mouse myeloma cell line expressing amyloidogenic light chain and found a significant reduction in light chain production without affecting cell viability. Similarly, small interfering RNA (siRNA) targeting amyloidogenic light chain production was able to inhibit the synthesis of light chain protein in vitro, and a single dose of electroporation-mediated siRNA delivery in vivo was demonstrated to effectively reduce circulating light chain levels in an AL light chain secreting plasmacytoma transplant animal model (44). These two studies provide a potential for targeting specific pathological light chain sequences, which may provide opportunities for personalized treatment strategies. Currently, RNAi is being developed for the treatment of TTR amyloidosis in clinical trials (NCT-01148953). Antisense oligonucleotides have been shown to be effective in suppressing TTR expression in an animal model and are currently entering clinical trials for treating TTR amyloidosis in humans (ISIS-TTRRx) (6).

Immunotherapy has also been intensively studied in amyloidosis. Notably, vaccinations targeting Aβ protein have been suggested to reduce amyloid fibril load in mice models of Alzheimer’s disease (14, 55, 110). Attempts have also been made to manipulate the immune response to accelerate fibril removal. Amyloid fibrils normally do not elicit a strong immune response, both because of their endogenous properties and the generally poor immunity of the patients (35). Over a decade ago, Hrncic et al. (45) reported that anti-light chain monoclonal antibodies in mice receiving human AL amyloidosis transplantation resulted in the rapid dissolution of amyloid fibrils and neutrophil infiltration. In addition to targeting light chain protein for removal, immunotherapy has also been used to target key proteins known to protect amyloid fibers from degradation. The glycoprotein serum amyloid P component (SAP) has been universally found in all types of amyloid fibril deposition, binding to motifs in the amyloid fibrils in a calcium-dependent manner (67, 99). SAP presence is thought to protect fibrils from proteolysis-mediated degradation (76). As such, by targeting SAP, one might directly disturb amyloid deposition. Using this concept, Bodin et al. (10) recently showed that antibodies targeting SAP in a systemic AA amyloidosis mouse model greatly accelerated the removal of massive visceral amyloid deposits via a complement-dependent and macrophage reaction without significant side effects. In a small study of humans with fibrinogen amyloidosis CPHPC, (R)-1-{6-[((R)-2-carboxy-pyrrolidin-1-yl]-6-oxa-hexa-noyl]pyrrolidine-2 carbonylic acid, a novel bis(o-proline), depleted SAP and resulted in an apparent stabilization of their disease and markedly slowed the progression of renal disease compared with historic controls (36).

These experimental approaches may represent viable therapeutic options, though they remain early in development and require further study before being evaluated in clinical settings.

Closing Remarks

Managing cardiac amyloidosis, particularly AL cardiac amyloidosis, has been a daunting task for clinicians, particularly given the rapid disease progression, reduced therapeutic options, and poor survival. Both basic research and clinical observation have provided compelling evidence for the direct cardiac toxicity of precursor amyloidogenic light chain proteins and have suggested the elimination of precursor proteins via either chemotherapy or stem cell transplantation to be a central goal in the management of AL amyloidosis. Newly introduced chemotherapeutic reagents and regimens coupled with the disease-staging system have also been shown to greatly enhance disease management in patients. As a result, the four-year overall survival in AL amyloidosis patients from date of diagnosis has significantly improved over the past three decades: 21% in 1977–1986, 24% in 1987–1996, and 33% in 1997–2006 (53). While significant advances have been made in the past decade, the overall prognosis associated with AL cardiomyopathy is still very poor. The high one-year mortality in AL amyloidosis patients suggests that early diagnosis of AL cardiomyopathy is paramount for the ultimate survival of patients. The identification and development of effective treatments demands a clear understanding of the molecular mechanisms that promote disease pathogenesis. Recent work has finally begun to make important inroads into understanding amyloid cardiomyopathy (particularly AL cardiomyopathy) and will lead to new potential treatments. Many of these treatments have entered early stages of development and will require close collaboration between basic scientists and clinical investigators to make effective treatment strategies a reality.
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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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Review

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