The Detrimental Effect of Mitochondrial Dysfunction On The Onset Of ATTR Amyloidosis

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Abstract

Transthyretin amyloidosis (ATTR) is a progressive and rare disease that is caused by a buildup of amyloid deposits of misfolded transthyretin (TTR) proteins in vital body organs such as the heart. Hepatocytes in the liver are the primary producers of these proteins. Groups of abnormal and misfolded TTR proteins called fibrils build up in the heart's left ventricle causing difficulty in pumping blood to the rest of the body. The endoplasmic reticulum (ER) consists of a network of membranes in a cell's cytoplasm that is the location of protein folding, and its dysfunction is implicated in ATTR pathogenesis. ER stress occurs when some factor leads to the accumulation of misfolded proteins. When stress in the ER increases, it can lead to energy depletion in the mitochondria.

Mitochondria are membrane-bound organelles that generate chemical energy that the cell needs to function in the form of adenosine triphosphate (ATP). Mitochondrial dysfunction is a blanket term used to denote aberrant mitochondrial activity. ER stress is known to promote mitochondrial dysfunction and vice versa. However, in the context of ATTR, the link between mitochondrial dysfunction and ER stress is not entirely defined. Our current hypothesis is that mitochondrial dysfunction contributes to ER stress, which is a principal mediator of ATTR. Herein we will discuss the mechanistic links between ER stress and mitochondrial dysfunction in ATTR pathogenesis.

Introduction

Transthyretin amyloidosis (ATTR) is a rare heart disease and is an under-recognized cause of major heart failure in adults [1]. This progressive disease is a buildup of amyloid deposits of transthyretin (TTR) proteins in the heart and other major organs. The symptoms of ATTR range in severity, from fatigue to dangerous arrhythmias. Due to a lack of knowledge and diagnostic methodologies, ATTR amyloidosis is commonly misdiagnosed, as up to 15% of older adults with heart failure may have unrecognized ATTR [1]. A recent study has also shown that ATTR amyloidosis has a prevalence rate of nearly 20% in a cohort with heart failure [2].

TTR is a protein that transports the thyroid hormone, thyroxine, and retinol-binding protein (RBP4) to various body parts [3]. Hepatocytes, the functional cells of the liver, are major producers of TTR, and recent evidence implicates the endoplasmic reticulum (ER) of hepatocytes as significant mediators of ATTR pathogenesis. The ER plays a vital role in protein folding, consisting of a membranous network of enzymes that assist in the folding and packaging of amino acid chains. After proteins are translated from mRNA strands in the ER, specific folding enzymes modify specific amino acids through editing mechanisms, which configure/fold proteins into the “native” 3D form [4]. Irreversible misfolding of defective transthyretin proteins leads to the deposition of these proteins in organs. Excess protein misfolding can also induce ER stress and ER stress responses, leading to resolution or further cellular dysfunction. A consequence of chronic ER stress is mitochondrial dysfunction, which will be further discussed in the Mitochondrial Stress section of this paper [5].

Mitochondria are membrane-bound organelles that play an essential role in the production of chemical energy in the form of ATP. Mitochondrial dysfunction occurs when there is abnormal activity in energy production and can lead to irregular functioning in other cellular organelles such as the endoplasmic reticulum (ER). The accumulation of mtDNA mutations and increased ROS production causes oxidative damage to macromolecules and organelles [6]. This, in turn, leads to reduced respiratory chain activity and ATP generation, all of which occur in the mitochondria. Another cause of mitochondrial dysfunction is biological aging.

Regarding ATTR, There is clear evidence that ATTR amyloidosis is a disease that has an increased risk of occurrence among older adults. For example, autopsies in people greater than the age of 80 years reported the prevalence of ATTR to be nearly 25%. In addition to this, the median age of ATTR-wt diagnosis is greater than 70 years [7]. This leads to the potential link of increased risk of ATTR as age increases. Because aging is a major contributor to mitochondrial dysfunction, and mitochondrial dysfunction may induce ER stress, we suspect that a link exists between ER stress and aging-associated mitochondrial dysfunction in ATTR pathogenesis. Herein, we provide a comprehensive overview of the potential link between ER stress and mitochondrial dysfunction, ATTR amyloidosis.

Transthyretin Amyloidosis (ATTR)

Transthyretin Amyloidosis, or ATTR, is an uncommon heart condition caused by the buildup of amyloid deposits of misfolded transthyretin (TTR) proteins in vital body organs such as the heart [2]. *Amyloidosis* refers to the disease caused by a buildup of abnormal amyloid proteins in the body's organs and nerves. ATTR amyloidosis is primarily caused by a protein called transthyretin that changes shape through misfolding in the endoplasmic reticulum (ER) and forms fibrous lumps that are deposited in various organs [8]. This condition can be inherited but more commonly is caused by deterioration of cell organelle function due to aging.

ATTR amyloidosis is a protein-folding disorder that begins its pathogenesis in the liver. Transthyretin (TTR) is a tetrameric transport protein that transports the thyroid hormone thyroxine and the retinol-binding protein (RBP) bound to retinol [9]. In healthy individuals, the ratio of RBP: to TTR in plasma is around 0.3 [13]. In humans, about 90% of plasma TTR is secreted from the liver, ranging from 20-40 mg/dl. TTR proteins have a tetramer structure and a globular or spherical shape consisting of four identical subunits. A tetramer structure consists of four subunits. The four monomers in the tetramer interact through non-covalent bonds. TTR is initially produced as a monomer and then assembled as a tetramer in the ER where it undergoes post-translational modifications [11]. The predominance of the β-chain structure in the polypeptide chains of TTR and the organization of β-sheets predispose to the formation of fibrils, ultimately causing transthyretin amyloidosis (ATTR) [10]. Hepatocytes in the liver secrete

TTR tetramers that dissociate into monomers and misfold into proteotoxic fibrils that deposit in target tissues, such as cardiac tissue or amyloid [8]. Dissociation of the tetramers leads to partial monomer unfolding, aggregate formation, and, ultimately, amyloid fibril assembly. Decreased folding efficiency in the ER and reduced export of the proteins may be caused by mutations in the monomeric chain or decreased cellular function induced by aging [12].

Endoplasmic Reticulum Stress

TTR proteins follow a protein-formation pathway that goes through the endoplasmic reticulum (ER), the cell's largest organelle [14]. The ER plays a critical role in the synthesis of TTR and ensures quality control of protein folding. The ER can be either smooth or rough. The rough ER contains ribosomes, which are small, round organelles that make and fold proteins [51]. The rough ER aims to guarantee that proteins are correctly folded and transported [15]. Helper molecules and other ER proteins perform these quality-control roles. Many folding factors that allow for the proper folding of proteins are characterized by a notable capacity to bind calcium [16]. With an influx of calcium (Ca^{2+}) in the ER lumen, Ca^{2+} binding proteins such as calreticulin and calnexin begin to function in the protein folding pathway once there is a high concentration of Ca^{2+} [17].

Since the function of the ER requires high calcium concentrations, the depletion of Ca^{2+} results in the deterioration of the proteins and triggers the unfolding protein response system (UPR). The UPR is activated when unfolded or misfolded proteins accumulate in the endoplasmic reticulum. Activation of the UPR involves three signaling pathways: Ire1, PERK, and ATF6 [18]. In response to an accumulation of unfolded proteins in the ER, three ER transmembrane proteins are activated- inositol-requiring enzyme 1 (IRE1), PRKR-like ER kinase (PERK), and activating factor 6α (ATF6α). These proteins initiate a signaling and transcriptional network leading to the UPR [19]. Most thermodynamically and kinetically unstable TTR variants, such as D18G and A25T TTRs, are recognized by these quality control pathways, which leads to protein degradation through ER-associated degradation (ERAD).

Essentially, when misfolded proteins attempt to exit the ER, the UPR recognizes the misfolded proteins, leading to the induction of ERAD. ERAD is a mechanism by which slowly folding or

misfolded proteins are cleared from the ER and degraded. [20] Once the UPR detects the misfolded proteins and signals the ERAD, the ERAD immediately expands the ER membranes and inhibits further translation. Additionally, misfolded proteins can be recruited to the ERAD system via ER chaperones or helper proteins for cytosolic degradation. Usually, the misfolded proteins are tagged for degradation through ubiquitin, a small regulatory protein [21]. After retrotranslocation into the cytosol, the proteins are degraded by the proteasomes in the cytosols [20].

The failure of the ERAD mechanism to degrade accumulated mutant proteins can induce ER stress, leading to cellular toxicity. It can also deprive the cells of important functional proteins required by other organelles such as the mitochondria [22]. Some moderately unstable amyloidogenic TTR variants may escape the ERAD system and be secreted at levels that can induce amyloidosis. The misfolded proteins can remain undetected by the ERAD system by presenting functional exit signals, as do the corresponding correctly folded proteins [22]. A compromised ERAD increases the forward trafficking of misfolded proteins containing ER exit signals.

The misfolding and aggregation of transthyretin (TTR) are known to be responsible for the development of amyloid transthyretin (ATTR) amyloidosis. The continuation of the production of unstable TTR variants can cause misfolded proteins (TTR monomers) to accumulate in the ER, resulting in prolonged ER stress. With more than 130 pathogenic mutations of the TTR gene, the mutant variants can destabilize and dissociate TTR tetramers into unfolded monomers and dimers [22]. Usually, the thermodynamically and kinetically unstable TTR variants in the liver are controlled through the ERAD. However, if the variants escape the degradation system of the liver, there may be increased secretion of defective amyloidogenic proteins, leading to increased deposits in organs such as the heart. The buildup of proteins through this mechanism leads to ATTR amyloidosis [8].

Mitochondrial Stress

The ER and mitochondria are dynamic organelles that interact physiologically and functionally [24]. One of the most critical aspects of their interactions is calcium signaling between the two organelles. When the ER forms close contact with nearly 20% of the mitochondrial surface, the ER communicates with the mitochondria through mitochondrial-associated membranes (MAM) [24]. The overlapping regions between the ER and mitochondria, MAMs, are zones that have pivotal roles in ensuring the efficient transmission of Ca^{2+} from the ER to the mitochondria. Their function is to stimulate oxidative metabolism and, conversely, enable the mitochondria to regulate ER Ca^{2+} homeostasis [25]. Additional important sites between the mitochondria and ER that regulate calcium homeostasis are the Mitochondria-Endoplasmic-Reticulum Contact sites or MERCs [26]. These sites consist of tight contacts with a distance shorter than 50 nm between the ER and mitochondrial membranes [27]. At MERCS, Ca^{2+} ions are transferred between the ER and mitochondria through a core protein complex [28]. One of these groups includes the IP3R/Grp75/VDAC proteins. IP3R is one of the most important calcium channels integrated into the ER membranes. This intracellular protein controls the release of Ca^{2+}, affecting cellular metabolism [29]. The VDAC protein is located in the outer mitochondrial membrane and mediates the uptake of Ca^{2+} by mitochondria. It functions as a gatekeeper for the entry and exit of mitochondrial metabolites [30]. Grp75 binds to IP3R and VDAC to improve the stability of the interaction of the two proteins, resulting in increased efficiency of Ca^{2+} transfer. The sigma-1 receptor, or Sig-1R, is a chaperone located on MAMs and also affects the transport of calcium ions by IP3R to increase the production of ATP, a source of cellular energy [29]. By binding to BiP, a chaperone found in the ER lumen, the complex with BiP and Sig-1R controls specific signaling stability to regulate Ca^{2+} signaling and ER stress [31].

Several ER chaperones, specifically, calreticulin, calnexin, CRP78/BiP, and FRP94, need an optimal calcium concentration for protein folding activity [32]. A decrease in calcium levels may inhibit protein folding in the ER. Specifically, a decrease in mitochondrial function would inhibit the transportation of Ca^{2+} ions through MAMs, which would in turn reduce protein folding or alternatively increase the likelihood of misfolded proteins, leading to the deposition of irregular transthyretin proteins in the heart [33].

The mitochondria consist of a double-membrane system; an intermembrane space separates the inner and outer mitochondrial membranes. The inner membrane forms numerous folds known as cristae, extending into the interior or matrix of the mitochondria [34].
Reactive Oxygen Species (ROS) are generated during mitochondrial oxidative phosphorylation [35]. They are mainly produced in the electron transport chain, a process in which electrons are transferred to reduce oxygen input through a series of redox reactions [36]. When electrons are transferred through the protein complexes, oxygen acts as a final electron acceptor in the ETC, forming water (H2O) [36]. However, when electrons prematurely leak from the ETC and react with oxygen, it results in the generation of ROS [37]. ROS plays an important role in the mitochondria as it regulates signaling pathways and allows for the transfer of ions between organelles.

In the mitochondria, Ca^{2+} plays specific roles in different compartments. For example, calcium ions in the intermembrane space (IMS) regulate the functions of many inner membranes (IMM) resident enzymes. Ca^{2+} is required in the mitochondrial matrix for ATP production via the Citric Acid Cycle and electron transport chain (ETC), both processes that generate energy [38]. It is known that the ER imports ATP and uses energy from ATP hydrolysis for protein folding [39]. Due to aging, there is an increased risk of the accumulation of reactive oxygen species (ROS) damaged mitochondrial DNA and proteins. ROS are natural byproducts of cellular respiration which occurs in the mitochondria. They play important roles in cell survival, and signaling, and induce cell differentiation and apoptosis. Specifically, ROS causes oxidative modification of each of the major cellular macromolecules (lipids, carbohydrates, DNA, proteins) [40]. ROS thus contributes to the natural aging process [41].

Aging is a progressive erosion of homeostatic equilibrium within cells, tissues, and organs of the body. Although the distinct mechanisms that catalyze biological aging are not entirely clear, mitochondrial dysfunction is a common theme observed in aged individuals. Mitochondrial dysfunction is a blanket term used to characterize abnormalities in the assembly, functionality, and degradation of damaged mitochondria. A principal consequence of mitochondrial dysfunction is a significant shift in their efficiency to produce ATP, with a concurrent increase in the mitochondrial ROS release.

Increased ROS can influence ATP production by directly damaging components of the electron transport chain or indirectly by modulating the metabolic intermediates necessary for ATP production, including TCA metabolites and cofactors like Ca^{2+}. Since mitochondrial processes are interdependent, a single abnormality can perturb other mitochondrial processes. For example, ROS-induced decline in ATP production shifts mitochondrial mobilization of Ca, and this may impact the stability of MERC [42].

During aging, damaged mitochondria produce less ATP, and more ROS accumulate [43]. The accumulation of ROS causes oxidative damage to macromolecules, including proteins. Although the exact mechanism of oxidative stress-inducing aging is not clear, the accumulation of ROS leads to the reduction of Ca^{2+} -ATPase activity, reducing Ca^{2+} transportation to the ER [40]. The ER uses calcium ions for several cellular processes, primarily in ER protein chaperoning and maturation [44]. Significant depletion of Ca^{2+} ions can inhibit protein folding activity in the ER or cause abnormal folding, accumulating misfolded proteins [25].

Additionally, the abnormal electron transport chain function induces mitochondrial dysfunction, directly decreasing Ca^{2+} and ATP production, which is crucial for many cellular processes [45]. The energy released by the ETC from the mitochondrial oxidative respiratory chain is used to create a proton gradient which drives ATP synthesis and creates a driving force of Ca^{2+} absorption. Through MAMs, Ca^{2+} is transferred directly from the ER to mitochondria and controls key mitochondrial functions [46]. With aging, the mechanism by which the mitochondria use up calcium ions and create ATP deteriorates. ATP enters the ER lumen through a cytosolic Ca^{2+}-antagonized mechanism [47]. Due to mitochondrial dysfunction, the MAMs will be unable to maintain homeostasis of calcium ions and the ER will also be dysfunctional due to the depletion of ATP energy resources. With a decrease in ATP supply, the ER cannot use calcium ions for the enzymes to function, leading to dysfunction of the UPR and ERAD systems, allowing the misfolded TTR protein variants to escape.

Therefore, if there is excessive ER stress and/or mitochondrial dysfunction, the imbalances will lead to protein misfolding and the deposition of misfolded proteins, inducing ATTR amyloidosis.

Conclusion/Discussion

Transthyretin amyloidosis (ATTR) is an uncommon disease characterized by the accumulation of amyloid deposits composed of misfolded transthyretin (TTR) proteins in vital body organs like the heart. The liver's hepatocytes are the primary producers of these proteins. This scientific review focuses on the mechanistic links between ER stress and mitochondrial dysfunction (and aging) in ATTR pathogenesis. The ER consists of a network of membranes in a cell's cytoplasm that is the location of protein folding, and its dysfunction is implicated in ATTR pathogenesis. ER stress occurs when some factor leads to the accumulation of misfolded proteins. When stress in the ER increases, it can lead to energy depletion in the mitochondria.

Mitochondria are membrane-bound organelles that generate chemical energy that the cell needs to function in the form of adenosine triphosphate (ATP). ER stress is known to promote mitochondrial dysfunction and vice versa. The interplay between the two organelles involves several key mechanisms.

1. Calcium Ion Depletion: A significant depletion of Ca^{2+} ions can inhibit protein folding activity in the ER or cause abnormal folding, accumulating misfolded proteins.
2. ATP Depletion: With a decrease in ATP supply, the ER cannot use calcium ions for the enzymes to function, leading to dysfunction of the UPR and ERAD systems, allowing the misfolded TTR protein variants to escape.

Through the depletion of the Ca^{2+} ion supply and the reduction of ATP production, there is a clear link between aging and protein misfolding, leading to ER Stress. Mitochondrial dysfunction plays a significant role in depositing misfolded transthyretin protein in the heart.

While questions regarding the roles of mitochondria and ATTR pathogenesis remain unresolved, the causal link between mitochondrial dysfunction, ER stress, and the onset of ATTR amyloidosis is clear. Mitochondrial dysfunction creates a domino effect, instigating the onset of ATTR amyloidosis through a cascade of events. Evidently, abnormalities in the mitochondria are linked to ER stress, which can lead to more protein misfolding. The buildup of misfolded proteins leads to the accumulation of TTR protein variants in the heart, stimulating the onset of

ATTR amyloidosis. The unmet needs of affected individuals require urgent attendance. Proposing further research methods to reveal the precise molecular mechanisms that underlie this relationship can help develop targeted therapies for ATTR, propelling breakthroughs in the scientific community for the treatment of ATTR amyloidosis.

Future Research

For future research, we recommend a focus on analyzing blood samples from patients diagnosed with ATTR amyloidosis. An in-depth analysis focusing on the protein levels of these samples may provide valuable insights into the specific molecular and cellular events associated with the disease and help identify novel diagnostic and therapeutic targets (Table 1).

Furthermore, examining the potential role of biomarkers in blood samples may facilitate the development of more accurate diagnostic methods, allowing us to monitor disease progression and treatment response. The field of research holds promise for advancing our understanding of ATTR and improving diagnosing strategies for affected individuals.

Table 1: Future Research Proposals for Diagnosis of ATTR Amyloidosis

Tissue/Organelle	Assay/Technique	Functional Readout
ATTR Cardiomyocytes Mitochondria	Proteomics, mtDNA copy number	Cardiac muscle cells, or cardiomyocytes, are striated, branched, and contain many mitochondria. These muscles contract rhythmically without rest. In ATTR amyloidosis, the cardiomyocytes have more buildup of excessive transthyretin proteins. The amyloid protein deposits cause the heart muscle to become stiff, leading to heart weakness [48].
Blood plasma	Metabolomics	- Serum levels of TTR in healthy adults range from 18 to 45 mg/dL– dependent on factors such as age, race, nutritional status, and net state of inflammation

		- Elevated levels of TTR proteins in the bloodstream are associated with ATTR amyloidosis. As the misfolding of TTR proteins increases, they can be detected in the bloodstream and used for diagnostic purposes [49].
Mitochondrial Antioxidant Gene Expression (SOD2)	Microarrays, qPCR	The SOD2 gene is located within the mitochondrial matrix. This enzyme catalyzes the reaction of superoxide (O2-) to the less reactive hydrogen peroxide (H2O2). This facilitates the passive diffusion of hydrogen peroxide away from the mitochondrial matrix. When the mitochondria are under stress, SOD2 deficiency leads to excessive ROS production and contributes to oxidative stress. This would increase the buildup of misfolded TTR proteins [50].
Mitochondrial Cristae Architecture	Transmission electron microscopy	Mitochondria in mature cardiomyocytes are unique to those found in other tissue types. They are significantly more abundant in the heart. Mitochondria in striated muscles appear to be located within membranous compartments along the myofiber. As a result of excess oxidative stress, the mitochondria have evidence of oxidative damage to DNA and lipids, such as oxidized blood markers and membrane lipids. Loss of mitochondrial function also leads to diminished production of ATP [51].

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