Human in vitro disease modeling of ATTR cardiac amyloidosis

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Background: Amyloidosis is a systemic protein-folding disorder characterized by extracellular misfolded fibrillar protein aggregates that are insoluble and resistant to proteolysis, leading to progressive and vital organ damage [1]. Transthyretin amyloidosis (ATTR) is one of the primary amyloidosis types that affect the heart. Based on the TTR gene sequence, ATTR is further classified into wild-type ATTR (ATTRwt) and hereditary ATTR (ATTRv). Advanced stage TTR amyloid infiltration of the myocardium results in progressive thickening and stiffening of the ventricle wall, and symptoms of restrictive cardiomyopathy and congestive heart failure. Due to the lack of specificity of cardiac amyloidosis symptoms, misdiagnosis and delays in diagnosis are common, resulting in worsening symptoms, organ damage, and increased mortality [2]. Despite the development of several disease-modifying agents [3], current experimental cell and animal models do not adequately represent the human situation, thereby limiting disease understanding and therapeutic discovery.

Purpose: This project aims to develop an in vitro ATTR disease model, which is critical to define early disease stages and disease progression, map human disease-specific pathways, and screen for novel drugs or validating cutting-edge therapeutic strategies.

Methods: Recombinant WT-TTR and V122I mutated TTR (V122I) were prepared using E.coli protein production system. TTR purification and acid-mediated TTR-fibril were characterized by SDS-PAGE, Thioflavin T fluorescence measurement, and transmission electron microscope (EM). iPSC-derived cardiomyocytes (iPS-CMs) were differentiated using GiWi protocol as described [4,5]. iPS-CMs or HUVECs were seeded on TTR or TTR-fibril, and we measured beating rate, cell viability (AlamarBlue and LiveDead), and cell/fibril morphology using immunofluorescence.

Results: Purified recombinant WT and V122I TTR was successfully generated (Figure 1A). Fluorescence increase (Thioflavin T) and EM revealed the presence of TTR-fibril (Figure 1B). iPS-CMs seeded on TTR-fibril resulted in a dose- and time-dependent increase in cell death. Confocal microscopy revealed the attachment of TTR-fibril on iPS-CMs, leading to sarcomere disarray, loss of cell-to-cell coupling, resulting in irregular beating (Figure 2A). Additionally, we found drastically affected viability of HUVECs upon TTR-fibril exposure (Figure 2B).

Conclusion: Here, we demonstrate an established method to generate purified TTR and TTR-fibril. TTR-fibril attached to the surface of iPS-CMs decreased cell viability and functionality. Similar reduction in HUVEC viability were observed as well. These findings reflect the cytotoxicity of TTR-fibril in a human in vitro model.

TTR and TTR-fibril characterization
Cell viability up TTR-fibril exposure