Published in final edited form as:
Autophagy. 2008 ; 4(6): 821–823.

Protein Misfolding and Cardiac Disease:

Establishing Cause and Effect

J. Scott Pattison and **Jeffrey Robbins***
Department of Pediatrics, Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229-3039

Abstract

Numerous neurodegenerative diseases are characterized by the accumulation of misfolded amyloidogenic proteins. Recent data indicate that a soluble pre-amyloid oligomer (PAO) may be the toxic entity in these diseases and the visible amyloid plaques, rather than causing the disease, may simply mark the terminal pathology. In prior studies, we observed PAO in the cardiomyocytes of many human heart failure samples. To test the hypothesis that cardiomyocyte-restricted expression of a known PAO is sufficient to cause heart failure, transgenic mice were created expressing polyglutamine repeats of 83 (PQ83) or 19 (PQ19).Long PQ repeats (>50) form PAOs and result in neurotoxicity in Huntington's disease, whereas shorter PQ repeats are benign. PQ83 expression caused the intracellular accumulation of PAOs and aggregates leading to cardiomyocyte death and heart failure. Evidence of increased autophagy and necrosis accompanied the PQ83 cardiomyocyte pathology. The data confirm that protein misfolding resulting in intracellular PAO accumulation is sufficient to cause cardiomyocyte death and heart failure.

Keywords

Protein misfolding; amyloid oligomer; heart disease; autophagy; heart; polyglutamine

Background

Heart failure (HF) is a leading cause of death in the US, affecting between 2–3 million Americans. HF is a common terminal endpoint in many cardiac diseases that stem from diverse etiologies including coronary artery disease, hypertension, and idiopathic and familial cardiomyopathies.[1, 2] Many cardiomyopathies are caused by mutations in cardiac sarcomeric, cytoskeletal, and associated proteins.[1] These mutations, as well as epigenetic stresses, can promote or cause protein misfolding, which can have diverse effects on that and other proteins' function, interactions, localization, steady state levels and turnover.[4,5] Some myopathic mutations are associated with known chaperones. For example mutations in desmin or its chaperone αB-cystallin, a small heat shock-like protein, can cause a desmin-related myopathy (DRM).[5–7] DRMs are characterized by insoluble intracellular proteinaceous accumulations of the mutant protein and its interaction partners ultimately resulting in distil muscle weakness and in a dilated cardiomyopathy.[5–8] This protein misfolding results in intracellular dysgenesis of the cardiac sarcomeres and hence is unique and can be distinguished from the misfolded

*Correspondence to: Jeffrey Robbins, MLC 7020, 240 Albert Sabin Way, Cincinnati, OH, 45229-3039 USA; Tel.: 513.636.8098; Fax: 513.636.5958; E-mail: jeff.robbins@cchmc.org.

Addendum to: ***Cardiomyocyte Expression of a Polyglutamine Pre-amyloid Oligomer Causes Heart Failure*** J.S. Pattison, A. Sanbe, A. Maloyan, H. Osinska, R. Klevitsky and J. Robbins Circulation 2008 117: 2743-2751

protein diseases associated with muscle and/or cardiac tissue, resulting in extracellular amyloidoses.

The accumulation of misfolded proteins into intracellular or extracellular aggregates is a common characteristic of the neurodegenerative amyloidoses including Alzheimer's, Huntington's and Parkinson's diseases.[9,10] As noted above, recent data suggest that the insoluble aggregates may not be inherently pathogenic; instead the toxic entity appears to be the soluble intermediates of fibril formation known as PAO.[11–14] These soluble oligomers share a common conformation-dependent protein structure, regardless of genetic sequence[15,16] and can be detected by conformation-specific antibodies.[7,16] Staining of mouse models of DRM and human HF samples for PAOs demonstrate that cardiomyocytes from diverse cardiomyopathies accumulate PAO, whereas healthy control hearts do not.[2] The presence of PAOs in heart-failure patients implies that there may be common pathogenic mechanisms between the neurodegenerative amyloidoses and at least some cardiomyopathies that result in end-stage HF. However, the direct relationship between intracellular PAO generation, protein aggregate deposition and cardiac disease pathology remains unclear.

Can PAO Expressed in Cardiomyocytes Cause Heart Failure?

To test the hypothesis that PAOs are intrinsically pathogenic upon expression and intracellular accumulation in cardiomyocytes and sufficient to cause cardiomyopathy, we generated transgenic mice with cardiomyocyte autonomous expression of an ectopic peptide containing83 repeats of glutamine (PQ83), which is causative in Huntington's disease. Parallel lines of mice that expressed equal or higher amounts of a non-amyloidogenic peptide containing only 19 repeats (PQ19) were created in order to ensure that high levels of an ectopic protein were not responsible for any resulting pathology.[3] If intracellular expression and accumulation of PAO is inherently toxic, expression of PQ83 should result in cardiomyocyte pathology, potential cell loss and subsequently HF.

Consistent with the hypothesis, expression of low levels of PQ83 peptide caused deposition of both PAOs and PQ83-containing aggresomes within the cardiomyocytes, leading to cardiomyocyte loss, progressive cardiac dilation and eventual death from HF by approximately 5–7 months. Further study of the observed cardiomyocyte death reveals increased autophagosomal activity and lysosomal content. Necrotic cells are apparent but apoptotic activation and markers of endoplasm reticulum stress could not be detected. Ultrastructural analysis finds a marked increase in the number of mutilamellar bodies and lysosomes as well as examples of double-membraned autophagic vacuoles engulfing the polyglutamine aggresomes. It is currently unclear whether the increases in autophagic and lysosomal content are beneficial or detrimental to cardiomyocyte survival during the development of PQ83-induced cardiomyopathy. Further studies are ongoing to determine whether autophagy is contributing to PAO-induced cardiomyocyte toxicity and death and at which stage of the disease.

Evaluating Potential Roles of Autophagy in Heart Failure

Autophagy is a critical pathway for the clearance of misfolded and aggregated proteins. Autophagy can function as a form of programmed cell death in some models but can be instrumental in cell survival during cellular starvation.[18,19] Autophagic dysregulation clearly contributes to cellular pathogenesis in many cell types, including the heart.[18,20] However, autophagic processes are also essential for basal cardiomyocyte function.[21,22] It is clear that ablation of autophagic or lysosomal function can, under some circumstances, be detrimental to the heart.[21,23,24] However, gross upregulation of autophagic/lysosomal function could be deleterious as well.[25,26] Thus, as is the case for any essential cellular function, cardiomyocyte-based autophagy likely requires finely tuned control so that homeostasis is maintained.

It remains to be determined if PAOs can be degraded by lysosomes independently from the non-soluble aggregates. Many data show that lysosomes can consume aggregates and, conversely, inhibition of lysosomal function can increase protein aggregate content in cells.[22,27] In some neurodegenerative models, autophagic induction is protective against toxic intracytosolic aggregate-prone proteins, whereas other evidence suggests that sequestering toxic amyloidogenic proteins into aggregates may prevent their cytotoxic actions.[20,28,15] These issues have not been rigorously addressed in human disease and whether the observed PAO accumulation in human HF samples is due to improper protein folding, insufficient or compromised degradative machinery, or ineffective delivery to the degradation apparatus is presently unclear.

Is protein misfolding and PAO a generalized pathway in the development of human HF? In many tissues, cellular stress and aging can cause the accumulation of non-degraded proteins, as well as generating relatively high levels of misfolded proteins.[4] Hundreds or even thousands of proteins have the capacity to misfold and become amyloidogenic and, if not cleared, can be cytotoxic.[15] Further compromising the cardiomyocyte's ability to clear these accumulations is its post-mitotic state, a trait shared with many cells types affected in the neurodegenerative disorders. Future work will establish which catabolic process is rate-limiting, and test the effects of autophagic/lysosomal modulation on cardiomyocyte survival. The putative relationships between autophagic/lysosomal accretion and necrotic cardiomyocyte death also need to be determined. Finally, we need to define the means by which PAO accumulation induces cell death and what commonalities, if any, are present as a result of PAO generation from different proteins and stresses.

References

1. Adams KF Jr. New epidemiologic perspectives concerning mild-to-moderate heart failure. Am J Med 2001;110:6S–13S. [PubMed: 11334770]
2. Lloyd-Jones DM, Larson MG, Leip EP, Beiser A, D'Agostino RB, Kannel WB, Murabito JM, Vasan RS, Benjamin EJ, Levy D. Lifetime risk for developing congestive heart failure: The Framingham heart study. Circulation 2002;106:3068–3072. [PubMed: 12473553]
3. Chien KR. Genotype, phenotype: Upstairs, downstairs in the family of cardiomyopathies. J Clin Invest 2003;111:175–178. [PubMed: 12531871]
4. Knoll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, Hayashi T, Shiga N, Yasukawa H, Schaper W, McKenna W, Yokoyama M, Schork NJ, Omens JH, McCulloch AD, Kimura A, Gregorio CC, Poller W, Schaper J, Schultheiss HP, Chien KR. The cardiac mechanical stretch sensor machinery involves a z disc complex that is defective in a subset of human dilated cardiomyopathy. Cell 2002;111:943–955. [PubMed: 12507422]
5. Wang X, Osinska H, Klevitsky R, Gerdes AM, Nieman M, Lorenz J, Hewett T, Robbins J. Expression of R120G-αB-crystallin causes aberrant desmin and αB-crystallin aggregation and cardiomyopathy in mice. Circ Res 2001;89:84–91. [PubMed: 11440982]
6. Sanbe A, Osinska H, Saffitz JE, Glabe CG, Kayed R, Maloyan A, Robbins J. Desmin-related cardiomyopathy in transgenic mice: A cardiac amyloidosis. Proc Natl Acad Sci U S A 2004;101:10132–10136. [PubMed: 15220483]
7. Wang X, Osinska H, Dorn GW 2nd, Nieman M, Lorenz JN, Gerdes AM, Witt S, Kimball T, Gulick J, Robbins J. Mouse model of desmin-related cardiomyopathy. Circulation 2001;103:2402–2407. [PubMed: 11352891]
8. Wang X, Klevitsky R, Huang W, Glasford J, Li F, Robbins J. αB-crystallin modulates protein aggregation of abnormal desmin. Circ Res 2003;93:998–1005. [PubMed: 14576194]
9. Kopito RR. Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 2000;10:524–530. [PubMed: 11121744]
10. Muchowski PJ, Wacker JL. Modulation of neurodegeneration by molecular chaperones. Nat Rev Neurosci 2005;6:11–22. [PubMed: 15611723]

11. Lue L-F, Kuo Y-M, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J. Soluble amyloid β peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol 1999;155:853–862. [PubMed: 10487842]
12. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL. Soluble pool of Aβ amyloid as a determinant of severity of neurodegeneration in alzheimer's disease. Ann Neurol 1999;46:860–866. [PubMed: 10589538]
13. Hsia AY, Masliah E, McConlogue L, Yu GQ, Tatsuno G, Hu K, Kholodenko D, Malenka RC, Nicoll RA, Mucke L. Plaque-independent disruption of neural circuits in alzheimer's disease mouse models. Proc Natl Acad Sci U S A 1999;96:3228–3233. [PubMed: 10077666]
14. Westerman MA, Cooper-Blacketer D, Mariash A, Kotilinek L, Kawarabayashi T, Younkin LH, Carlson GA, Younkin SG, Ashe KH. The relationship between abeta and memory in the tg2576 mouse model of alzheimer's disease. J Neurosci 2002;22:1858–1867. [PubMed: 11880515]
15. Glabe CG, Kayed R. Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. Neurology 2006;66:S74–S78. [PubMed: 16432151]
16. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 2003;300:486–489. [PubMed: 12702875]
17. Pattison JS, Sanbe A, Maloyan A, Osinska H, Klevitsky R, Robbins J. Cardiomyocyte expression of a polyglutamine preamyloid oligomer causes heart failure. Circulation 2008;117:2743–2751. [PubMed: 18490523]
18. Zhu H, Tannous P, Johnstone JL, Kong Y, Shelton JM, Richardson JA, Le V, Levine B, Rothermel BA, Hill JA. Cardiac autophagy is a maladaptive response to hemodynamic stress. J Clin Invest 2007;117:1782–1793. [PubMed: 17607355]
19. Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, Sadoshima J. Distinct roles of autophagy in the heart during ischemia and reperfusion: Roles of amp-activated protein kinase and beclin 1 in mediating autophagy. Circ Res 2007;100:914–922. [PubMed: 17332429]
20. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ, Rubinsztein DC. Inhibition of mTor induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of huntington disease. Nat Genet 2004;36:585–595. [PubMed: 15146184]
21. Nakai A, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M, Omiya S, Mizote I, Matsumura Y, Asahi M, Nishida K, Hori M, Mizushima N, Otsu K. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. Nat Med 2007;13:619–624. [PubMed: 17450150]
22. Williams A, Jahreiss L, Sarkar S, Saiki S, Menzies FM, Ravikumar B, Rubinsztein DC. Aggregate-prone proteins are cleared from the cytosol by autophagy: Therapeutic implications. Curr Top Dev Biol 2006;76:89–101. [PubMed: 17118264]
23. Stypmann J, Glaser K, Roth W, Tobin DJ, Petermann I, Matthias R, Monnig G, Haverkamp W, Breithardt G, Schmahl W, Peters C, Reinheckel T. Dilated cardiomyopathy in mice deficient for the lysosomal cysteine peptidase cathepsin l. Proc Natl Acad Sci U S A 2002;99:6234–6239. [PubMed: 11972068]
24. Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lullmann-Rauch R, Janssen PM, Blanz J, von Figura K, Saftig P. Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. Nature 2000;406:902–906. [PubMed: 10972293]
25. Vogler C, Galvin N, Levy B, Grubb J, Jiang J, Zhou XY, Sly WS. Transgene produces massive overexpression of human β-glucuronidase in mice, lysosomal storage of enzyme, and strain-dependent tumors. Proc Natl Acad Sci U S A 2003;100:2669–2673. [PubMed: 12591953]
26. Scott RC, Juhász G, Neufeld TP. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Curr Biol 2007;17:1–11. [PubMed: 17208179]
27. Lee HJ, Khoshaghideh F, Patel S, Lee SJ. Clearance of α-synuclein oligomeric intermediates via the lysosomal degradation pathway. J Neurosci 2004;24:1888–1896. [PubMed: 14985429]
28. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 2004;431:805–810. [PubMed: 15483602]

29. Terman A, Gustafsson B, Brunk UT. Autophagy, organelles and ageing. J Pathol 2007;211:134–143. [PubMed: 17200947]