Molecular Mechanisms of α-Crystallinopathy and Its Therapeutic Strategy

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α-B-Crystallin (CryAB, gene map locus: 11q22.3—q23.1) is a member of the small heat shock protein (HSP) family, a group of proteins that prevent protein aggregation upon exposure of a cell to heat and/or restore the biological activity of cell substrates. The missense mutation and the deletion mutation of CryAB can cause various forms of muscular disorder, including restrictive, hypertrophic, and dilated cardiomyopathies, heart failure, and skeletal muscle weakness. Collectively, these diseases constitute a rare autosomal-dominant inherited disorder called α-crystallinopathy (crystallinopathy), also known as desmin-related cardiomyopathy. The disease is a misfolded protein-related disease characterized by the formation of insoluble protein aggregates consisting of the CryAB protein in the patient’s cardiomyocytes and skeletal myocytes. The details of crystallinopathy are unclear at the present time; what has been discovered concerning the disease mechanisms underlying crystallinopathy has been through experiments with genetically modified mice such as the CryAB knockout mouse and various mutant CryAB transgenic (TG) mice. Crystallinopathy can be recapitulated in TG mice by expressing the mutant CryAB Arg120Gly (R120G) protein, a causal mutation of crystallinopathy, specifically in the cardiomyocytes. CryAB R120G causes perinuclear formation of aggresomes containing preamyloid oligomer intermediates, which are well-known as a primary toxic species in neurodegenerative disease. This suggests that crystallinopathy caused by the CryAB mutation could be considered one of the aggresomal and amyloid-related diseases. Moreover, recent findings have indicated that enhancement of HSP induction and inhibition of apoptotic cell death by mitochondrial protection may be a new therapeutic strategy for patients with crystallinopathy.

Key words heat shock protein; cardiomyopathy; amyloid

1. CRYAB AS A CHAPERONE PROTEIN

The proteins α-A-crystallin (CryAA, also known as HSPB4) and α-B-crystallin (CryAB, also known as HSPB5) were initially of interest as major structural proteins present in the lens of the vertebrate eye.1) The subsequent discovery that they were related to the small heat shock proteins (HSPs) in Drosophila2) prompted a reevaluation of their broader role(s). CryAB is now assigned to the small HSP family,3) which also includes HSP20 (HSPB6), HSP22 (HSPB8), HSPB2, HSPB3, and HSP27 (HSPB1).4) The small HSPs share sequence similarity within the α-crystallin domain but exhibit different patterns of gene expression, transcriptional regulation, and subcellular localization.5) The chaperone-like activity of the small HSPs, which are grouped together based on their ability to prevent protein aggregation and/or restore the biological activity of cell substrates, is widely believed to be a protective mechanism against protein misfolding and denaturation triggered by noxious environmental stimuli, such as hyperthermic stress, heavy metals, ischemic injury, and some genetic diseases.4–6)

Although CryAB was originally discovered and classified as a lens protein,7) it is also found in nonlenticular cell types, with cardiac and skeletal muscles containing the highest CryAB levels among the non-lenticular tissues.8) CryAB binds both desmin and cytoplasmic actin and possesses a molecular chaperone function in vitro.9,10) In vivo, CryAB exists as an oligomer, with mass spectrometry data indicating that the predominant species is a 28-subunit oligomer.11) Like other chaperones, CryAB responds to stressful conditions by binding to unfolded proteins and preventing their denaturation and aggregation.12)

2. α-CRYSTALLINOPATHY (α-B-CRYSTALLINOPATHY)

Myofibrillar myopathy (MFM) is an imprecise term that refers to a group of morphologically homogeneous but genetically heterogeneous chronic neuromuscular disorders.13–15) The morphologic changes in skeletal and cardiac muscles in MFM result from disintegration of the sarcomeric Z disc and the myofibrils, followed by abnormal ectopic accumulation of multiple proteins involved in the structure of the Z disc. This abnormal ectopic accumulation includes desmin, dystrophin, myotilin, sarcoglycans, neural cell adhesion molecule, plectin, gelsolin, ubiquitin, filamin C, Xin, TAR DNA-binding protein 43, and cochaperones including CryAB.13) The disease is usually transmitted by autosomal-dominant inheritance.13) Several MFM disease-causal genes have been identified, including desmin and CryAB.13)

Vicart et al.16) identified an arginine-to-glycine missense mutation (R120G) at amino acid position 120 in CryAB that co-segregates with the disease phenotype in the French pedigree of desmin-related MFM (also called desmin-related cardiomyopathy, DRM). Muscle cell lines transfected with the mutant CryAB cDNA develop intracellular aggregates that contain both desmin and CryAB, as observed in muscle fibers from these MFM patients.

Subsequently, two heterozygous truncating mutations, 464delCT and Gln151X, were observed in the CryAB gene in two patients,17) both of whom presented with symmetric proximal and distal muscle weakness starting in adulthood and with atrophy as well as respiratory involvement. Recently, another patient with the CryAB mutation (Gly145Ser) has been reported.18) This patient’s phenotype included slowly progressive distal leg weakness, intermittent atrial fibrillation, and abnormal accumulation of multiple proteins in...
A biopsy sample. The subset of MFM represented by these latter three patients is termed α-crystallinopathy (also α-B-crystallinopathy).

3. ANIMAL MODEL OF α-CRYSTALLINOPATHY AND ASSOCIATED DISEASE PHENOTYPE

In an in vitro assay using α-lactalbumin, alcohol dehydrogenase, and insulin as target proteins, a recombinant CryAB R120G protein was found to have partially or completely lost its capacity to function as a chaperone. Surprisingly, however, when the CryAB gene was knocked out in mice, a relatively benign phenotype was observed. These mice showed normal development, with no cataracts or cardiac abnormalities, although some degeneration of skeletal muscle occurred. It is not entirely clear from this experiment whether CryAB is necessary to maintain skeletal muscle cell integrity, given that both the CryAB gene and the adjacent HSPB2 were targeted in these mice. Thus, it is unclear whether a missense mutation alone can cause α-crystallinopathy due to the loss of CryAB function. In a subsequent attempt to study the relationship between the missense mutation of CryAB and cell structure and function by another means, transgenic (TG) mice expressing CryAB R120G were generated and compared with CryAB wild-type TG mice as a control. The phenotype of the CryAB R120G TG mouse includes the accumulation of CryAB aggregates, composed in part of desmin, as well as sarcomeric disorganization, hypocontractility and impaired relaxation, mild ventricular hypertrophy with marked chamber dilatation, and premature death at approximately 6 months of age. This phenotype indicates that the TG mouse exhibits features strikingly similar to those observed in α-crystallinopathy patients. Since overexpression of wild-type CryAB was relatively benign, with no increase in the mortality rate and no tendency toward cardiac disease, even in a line in which CryAB mRNA expression was increased 104-fold and the protein level was increased 11-fold, the data show that the R120G mutation can cause α-crystallinopathy on its own, is dominant negative, and results in cardiac hypertrophy.

CryAB can bind not only to desmin but also to contractile proteins such as actin and titin; in addition, it has been detected at the M-line, indicating that it interacts with myosin-2. Thus the CryAB R120G protein may retain the ability to interact with contractile proteins, and this interaction may be enhanced relative to the normal affinity for the protein, as is the case with FBX4, a member of the F-box family of proteins. This may provide a partial explanation for the pathogenicity of the mutant relative to the CryAB null; accordingly, a reasonable working hypothesis is that CryAB R120G, which is defective in chaperone activity, binds tightly to nascent contractile protein(s), preventing them from folding correctly, and integrating into productive sarcomeres. The presence of contractile protein fragments within the aggregates suggests that mutant CryAB binding may directly disturb contractile protein function, rendering the muscle particularly sensitive to the action of CryAB R120G. Thus the pathogenesis of this mutation cannot be ascribed to a simple loss or gain of function, but probably reflects a synergistic combination of both.

4. AGGRESOME AND AMYLOID OLIGOMER IN α-CRYSTALLINOPATHY

The CryAB R120G TG mouse exhibits accumulation of CryAB aggregates, including desmin, and sarcomeric disorganization in the heart. In general, pathological protein aggregates tend to be insoluble and metabolically stable under physiological conditions; their accumulation is linked to cellular degeneration and organ failure. Cells have an active system that responds to protein aggregation by sequestering aggregates into inclusion bodies termed “aggresomes.” Aggresomes are common cytopathological features in a number of neurodegenerative, protein aggregation-related pathologies. Proto-aggresomes are first detectable at various sites in the cytoplasm, though they rapidly accrete around the microtubule organizing center (MTOC) adjacent to the nucleus. This process is an active one and involves the binding of the proto-aggresomes to dynein motors and their subsequent retrograde transport along the microtubule network. Although the composition of aggrecosomes can vary, they usually contain components of the proteasome, molecular chaperones, ubiquitin conjugates, and intermediate filament proteins. The aggregates induced by CryAB R120G are sequestered in aggrecosomes in non-muscle cell lines as well as cardiomyocytes. Thus α-crystallinopathy may be considered one of the aggrecosomal diseases.

Amyloidosis is well characterized in many tissues, including the heart, and is generally thought of as a heterogeneous syndrome characterized by extracellular proteinaceous fibrils that can form insoluble deposits. These deposits, which are now known to include intracellular aggregates or inclusions, can be stained with amyloidphilic dyes such as Congo red. Many of the misfolded proteins responsible for or present in these aggregates have been identified. Both hereditary and non-hereditary forms of systemic amyloidosis have been identified and can have diverse effects on cardiac function, resulting in dilative cardiomyopathy, restrictive cardiomyopathy, or diastolic dysfunction. Accumulation and aggregation of misfolded proteins are a hallmark of the amyloidoses, which include Alzheimer’s disease, Huntington’s disease, Parkinson’s disease, and the spongiform encephalopathies. In many cases, proteinaceous aggregates form as a consequence of mutation, modification, or other alteration of the primary structure of the causative protein or proteins, disturbing their tertiary structures or post translational assembly into oligomers. A growing body of evidence suggests that fibrillar deposits may not be the primary toxic agent, but rather that the inherent pathogenicity resides in soluble oligomers that represent intermediates in the fiber formation pathway. These soluble oligomers can be formed from many different sequences, but they exhibit a common conformation-dependent structure. The presence of these prefibrillar intermediates can be detected by the oligomer-specific antibody. Sanbe et al. showed that this antibody intensely stains CryAB R120G TG hearts in a mouse model and that, strikingly, it reacts with a number of independent samples derived from human heart failure patients while showing little or no reactivity with samples derived from “normal” hearts, demonstrating that cardiomyocytes subjected to diverse cardiomyopathies contain structurally related amyloid oligomers of some type.
ence in heart failure patients of proteins related to the soluble oligomers yet formed by a variety of amyloidogenic proteins is intriguing and implies that there may be common pathogenic mechanisms between at least a subset of the degenerative amyloidoses and cardiomyopathic diseases.

5. MITOCHONDRIAL INJURY IN α-CRYSTALLINOPATHY

Cardiac mitochondrial organization and architecture as well as mitochondrial respiration appear to affect young adult CryAB R120G TG mice disproportionately before any reduction in cardiac function is observed. Disruption of the desmin network rapidly leads to alterations in mitochondrial positioning and structure in CryAB R120G TG mouse hearts, and similar observations were made in striated muscles.21) The desmin network rapidly leads to alterations in mitochondrial organization,21) a result consistent with the observations in the CryAB R120G TG mice.30) It is hypothesized that disturbances of the tight juxtaposition of mitochondria over the interiors of sarcomeres result in alterations in cellular metabolism. These data are consistent with the observation that CryAB R120G specifically associates with mitochondria through voltage-dependent anion channel (VDAC) interaction early in the pathogenic process. The significance of the preferential association of CryAB R120G, but not the normal protein, with the VDAC is unclear but raises the possibility that CryAB R120G may have a direct impact on either VDAC or a mitochondrial protein associated with the mitochondrial permeability transition pore (PTP). What is clear is that mitochondrial dysfunction is one of the earliest detectable events in the development of R120G-mediated cardiomyopathy and appears to play a major role in the development of pathology. Mitochondrial permeability transition is clearly affected in CryAB R120G-transfected cardiomyocytes and precedes any increase in the levels of apoptotic markers.28)

Another explanation of the mitochondrial dysfunction that results from CryAB R120G revolves around the amyloid oligomer. The connections between amyloid deposition, mitochondrial dysfunction, and cell degeneration and death remain contentious, but an increasing quantity of data links amyloidogenic proteins to mitochondrial toxicity. The exposure of isolated brain mitochondria to β-amyloid causes a decrease in mitochondrial enzyme activity, respiration, and membrane potential.51) β-Amyloid can also activate the PTP opening, resulting in mitochondrial swelling.52) A result consistent with the observations in the CryAB R120G TG hearts.20) Impaired function of complex I has also been linked to the development of Parkinson’s and Alzheimer’s diseases,53,54) while reduced levels of complex I in the cerebellum are associated with Down’s syndrome.55) In Parkinson’s disease, the proteins parkin and α-synuclein, which are components of the abnormal aggregates (Lewy bodies) found in Parkinson’s patients’ neurons, bind to one another in vitro, inhibiting the mitochondrial respiratory chain and thereby increasing incorporation of α-synuclein into the aggregates in vitro.56) Finally, deficits in energy metabolism have been proposed as a primary pathogenic mechanism in Huntington’s disease, with elevated lactate levels being detected in the occipital cortex and basal ganglia.57) Ultrastructural analyses demonstrated that mutant huntingtin appears to be present on neuronal mitochondrial membranes58) and can directly increase mitochondrial susceptibility to calcium-induced permeability transition, resulting in the release of cytochrome c.59) These results are consistent with the mechanisms that have been suggested to be involved in CryAB R120G pathogenesis, as the data show that CryAB R120G expression leads to detectable amyloid formation in cardiomyocytes and mitochondrial dysfunction,28) which in turn could contribute to more rapid amyloid accumulation and an inherently unstable feed-forward loop.

6. APOPTOTIC CELL DEATH IN α-CRYSTALLINOPATHY

Apoptosis, which is potentially important in heart failure, is well known to be activated by the release of cytochrome c.60–63) Wencker et al. established a causal role by showing that very low levels of myocyte apoptosis were sufficient to cause lethal dilated cardiomyopathy.62) In a retrospective study of 33 patients who had died of acute myocarditis, cardiomyocyte apoptosis was identified as a common mechanism of myocardial damage, with significantly more apoptotic cardiomyocytes present in patients who had died from progressive heart failure compared with those who had died suddenly from cardiac arrest.64) High levels of activated caspase-3 were detected in CryAB R120G TG hearts in the later stages of progressive heart failure, as was the presence of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL)-positive cardiomyocytes.65,66) This data point to the importance of a progressive pathology in the development of heart failure. First, it is clear that the expression of CryAB R120G has acute effects on cardiomyocyte mechanics, affecting contractility through as yet undefined mechanisms, although the accumulating aggregates could certainly play a physical role in attenuating normal cardiomyocyte contractile behavior. Alterations in contractility can be sensed by multiple mechanisms, resulting in global responses at the transcriptional and translational levels, but it seems that a crucial aspect of early heart failure pathology is linked to alterations in respiration.28) Mitochondria-sarcomere architecture is affected very early: complex I activity is significantly attenuated, with a 50% reduction occurring by 6 weeks, before any alterations in cardiac function can be detected.29) This becomes more severe over a period of 2—3 months, eventually leading to the release of cytochrome c and the activation of apoptosis. Thus these effects can eventually lead to the activation of apoptotic pathways that further compromise cardiomyocyte function and viability, resulting in the development of heart failure and death.

7. THERAPEUTIC STRATEGY FOR α-CRYSTALLINOPATHY

Induction of Small HSPs CryAB is a small HSP that probably acts as a molecular chaperone; such proteins are usually present as complexes, each with a partner such as HSPB1, HSPB8, or a target protein.67,68) This implies that small HSPs, such as HSPB1 and HSPB8, may modify the aggresomal formation of CryAB R120G.66) It was found that HSPB1 as well as HSPB8 can inhibit amyloid oligomer formation and aggresomal formation induced by CryAB R120G.
at the recombinant protein, cardiomyocyte, and in vivo heart levels (Fig. 1).66,69) Cardiac-specific TG mice expressing HSPB8 also show inhibited progression of cardiomyopathy compared with ordinary R120G TG mice.66) These results imply that enhancing the induction of small HSPs could be beneficial in the treatment of α-crystallinopathy. Furthermore, geranylgeranylacetone (GGA), a nontoxic antiulcer drug and an inducer of small HSPs,70) can induce the expression of HSPB8 and HSPB1 and reduce the formation of amyloid oligomers as well as insoluble aggregates in HSPB5 R120G TG mice.66) Treatment with GGA led to a reduction in heart size, inhibition of interstitial fibrosis, and recovery of cardiac function as well as improved survival.66) These results indicate that the induction of small HSPs will be beneficial in the treatment of α-crystallinopathy.

Exercise Results obtained from mouse models of neurodegenerative disease show that environmental enrichment or voluntary exercise is beneficial in terms of delaying the onset and progression of disease.71–74) Investigators modeled human physical, social, and intellectual enrichment by placing at least two animals in large cages with running wheels, toys, and colorful tunnels.75) The physical component of environmental enrichment has particular clinical importance: voluntary exercise delayed the onset of neurological deficits in a mouse model of Huntington’s disease,74) and long-term exercise decreased in neural amyloid deposits and enhanced learning ability compared with nonexercised littermates.71,72) On the basis of these data and the parallels between these neurodegenerative disorders and CryAB R120G-induced cardiomyopathy, it is hypothesized that CryAB R120G disease would also respond favorably to prolonged voluntary exercise, reducing heart failure symptoms and rescuing the mice from premature death.76) Six months of voluntary exercise in CryAB R120G animals resulted in 100% survival beyond the point in time when all unexercised mice had died. After 22 weeks of exercise, amyloid oligomer levels were 47% lower than in unexercised CryAB R120G control mice.76) Although CryAB R120G expression led to decreased levels of the metallo-membrane endopeptidase nepriyisin in unexercised CryAB R120G mice, normal levels were maintained in exercised mice of the same genotype.76) In vitro loss-of-function and gain-of-function experiments using adenovirus-infected cardiomyocytes confirmed the importance of nepriyisin in ameliorating amyloid oligomer accumulation by CryAB R120G.76) The data suggest that voluntary exercise slows the disease progression toward heart failure in CryAB R120G TG mice and that amyloid oligomer accumulation may be mediated, at least in part, by decreasing nepriyisin activity (Fig. 1).

Antiapoptotic Approaches The cellular toxicity induced by CryAB R120G-amyloid oligomers is probably associated with mitochondrial function as well as with the induction of apoptotic cell death due to the release of cytochrome c from mitochondria.28,66) It is uncertain, however, whether the inhibition of apoptotic cell death is sufficient treatment for α-crystallinopathy. A recent study has shown that sustained B-cell lymphoma 2 (BCL2) overexpression in R120G hearts prolonged mouse survival and was associated with fewer mitochondrial abnormalities, restoration of cardiac function, and attenuation of apoptosis.65) More recently, the protective effect of nicorandil, a mitoK(ATP) channel opener, on disease progression was observed in CryAB R120G TG mice: rates of mitochondrial injury and apoptotic cell death were concomitantly reduced as survival rate accordingly increased (Fig. 1).77,78) Treatment with mitoK(ATP) channel openers such as nicorandil may represent a new therapeutic strategy for patients with α-crystallinopathy.77,78) Yet the disease mechanisms underlying α-crystallinopathy do not consist of apoptosis alone: a recent study has demonstrated that both apoptotic and necrotic cell death were detected in R120G TG mouse hearts, and that necrotic cell death was actually upregulated when apoptotic signaling was inhibited.65) These results imply that the inhibition of apoptotic signaling resulted in the upregulation of autophagy and alternative death pathways, with the net result being increased necrosis.65) Thus, although the inhibition of apoptotic cell death prolonged life in this α-crystallinopathy model, in the absence of apoptosis, it is possible that another death pathway such as necrosis can be activated.65)
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