Serine Phosphorylation and Suppression of Apoptosis by the Small Heat Shock Protein αB-Crystallin

Keith A. Webster

A poptotic death of cardiac myocytes is a central feature of ischemic heart disease and a prime target for therapeutic intervention. Multiple stress factors are associated with ischemic stress and a battery of intrinsic pathways work to attenuate damage. These include antioxidants, antiapoptotic factors such as Bcl-2 proteins, and endogenous caspase inhibitors such as ARC (see review1). αB-crystallin is a relative newcomer to this defensive arsenal; it has strong antiapoptotic properties and, when fully induced, may constitute as much as 5% of total cardiac myocyte protein. As such, αB-crystallin may have critical structural as well as protective functions in the heart. The signaling pathways that regulate αB-crystallin activity and the molecular mechanism of protection are not understood and some aspects of these are presently controversial. In this issue of Circulation Research, Morrison et al.2 describe the involvement of a critical serine residue at position 59 of the αB-crystallin that is targeted by the p38β-MAPKAP-K2 pathway during ischemic stress. They show that phosphorylation of this serine may be the key step for the activation and regulation of the antiapoptotic function(s) of αB-crystallin during ischemia.

Crystallin Family Proteins

The crystallins are a family of dual function proteins responsible for the transparent and refractive properties of the eye lens. At least 11 vertebrate crystallins have been described and several of these have secondary chaperoning and antioxidant activities that serve to protect the lens from light-induced oxidative stress (see reviews3,4). αB-crystallin is also a small heat shock protein (smHSP) structurally related to other smHSPs including αA-crystallin, hsp27, hsp20, hsp22, myotonic dystrophy protein kinase binding protein, and HSPB3.5,6 All of these except αA-crystallin are abundantly expressed in muscle where the chaperone activity plays a key role in response to stress. αB-crystallin is also strongly expressed lung, kidney, brain, and heart. It is induced during myogenic differentiation and is first detected in the heart during development. The selective expression of αB-crystallin in muscle is probably mediated by E-box and SRE motifs contained in the αB-crystallin promoter that bind the myogenic factors MyoD and MEF-2, respectively.7 αB-crystallin monomers (≈25 kDa) aggregate into multimeric units of >600 kDa, and these aggregates may be necessary for the chaperoning functions.7 It has been proposed that chaperoning by αB-crystallin can stabilize myofilament proteins through selective interactions with desmin, titin, and nebullete.9,10 Ectopic overexpression of αB-crystallin protects cardiac myocytes from ischemic damage.4,9,11

Mechanism of Action and Molecular Regulation of αB-Crystallin

Multiple stimuli responding to thermal, osmotic, ischemic, and oxidative stress induce and activate αB-crystallin through both transcriptional and posttranslational pathways.12 Ischemia promotes the translocation of αB-crystallin from diffuse locations in the myoplasm to defined structures in the sarcomere (Z-band) and nucleus, and this translocation may be key to protection.9 Translocation is transient and correlates closely with changes in the phosphorylation and perhaps level of oligomerization of αB-crystallin units.5,13 Phosphorylation appears to promote dissociation of αB-crystallin oligomers and different groups have associated this with decreased or increased chaperoning activity and cytoprotection.5,9 At least part of the chaperoning functions of αB-crystallin is associated with conserved C-terminal peptides that are located on the exterior surface of the oligomeric structures. Mutation of the 2 C-terminal lysine residues to glycine reduces oligomer size by >50% and eliminates protection of cardiac myocytes during ischemia.13

Regulation by Serine-Phosphorylation

The influence of phosphorylation and the structure-function relationships of αB-crystallin are controversial and are subjects of the article by Morrison et al.2 In their article, Morrison et al provide new information into how αB-crystallin activity may be regulated during osmotic and ischemic stress. They demonstrate that a single serine residue at position 59 that is a target for the p38β-MAPKAP-2K pathway may be entirely responsible for the activation of αB-crystallin during ischemia. This result appears to be at variance with other reports on the effects of serine phosphorylation in other models.

Negative Regulation of smHSPs by Serine Phosphorylation in COS and Glioma Cells

Hsp27 is closely related to αB-crystallin, and like αB-crystallin, it is phosphorylated by p38-MAPK and protein kinase C pathways in response to stress and growth factors.14 Phosphorylation of hsp27 or pseudophosphorylation by re-
placement of the target serines with aspartate or glutamate causes dissociation of hsp27 oligomers and reduced stress tolerance. Under similar stress conditions αB-crystallin is phosphorylated on three serine residues including Ser-19, Ser-45, and Ser-59; Ser-45 and Ser-59 are substrates for the p44/42 ERK-MAPK and p38-MAPKAP-2 pathways, respectively. Phosphorylation of these αB-crystallin serine residues in COS and glioma cells was reported to mediate reduced oligomerization and decreased chaperone activity and thermotolerance.

Negative Regulation by Serine Phosphorylation in Skeletal Muscle

Native αB-crystallin plays an important antiapoptotic role during myogenic differentiation and development. This is reflected in several settings: a missense mutation of αB-crystallin (R120G) causes autosomal dominant myopathy with disruption of myofilaments and accumulation of desmin-crystallin (R120G) causes autosomal dominant myopathy. The R120G mutant is severely compromised in its chaperone activity. αB-crystallin protects skeletal myocytes against TNF-α and DNA damage–induced apoptosis by inhibiting the processing and activation of caspase 3. Ectopic expression of wild-type αB-crystallin but not the R120G mutant conveyed antiapoptosis to differentiating myocytes. Importantly, ectopic expression of a pseudophosphorylation αB-crystallin mutant with the 3 target serine residues substituted for asparagine was completely devoid of antiapoptotic activity. Neither the R120G nor the pseudophosphorylation αB-crystallin mutant was able to inhibit caspase 3. In these studies, it was concluded that serine phosphorylation at positions 19, 45, and 59 inactivated αB-crystallin possibly by dissociating aggregates.

Positive Regulation by Serine-Phosphorylation in Cardiac Myocytes

In contrast to reports that the antiapoptotic functions of αB-crystallin are inactivated by serine phosphorylation, Morrison et al report the opposite effect. In cardiac myocytes subjected to osmotic or ischemic stress, these authors demonstrate that rather than mediating a negative regulation, phosphorylation of Ser-59 is actually required to activate αB-crystallin. Caspase 3 again appears to be the target and phosphorylation of Ser-19 and Ser-45 were neutral in these assays. Morrison et al propose that Ser-59 is selectively targeted in cardiac myocytes by the MKK6-p38β-MAPKAP-K2 kinase cascade that is initiated by osmotic and ischemic stress (see Figure). By substituting each of the αB-crystallin serine residues individually or in combination with either alanine to block phosphorylation or glutamine to mimic constitutive phosphorylation, Morrison et al defined the contribution of each site to αB-crystallin activity. They demonstrate that αB-crystallin is inactive when Ser-59 is substituted by Ala, and fully active when Ser-59 is replaced by Glu. Neonatal cardiac myocytes expressing the Ala substitution at Ser-59 were 3-fold more susceptible to osmotic and ischemic stress, whereas myocytes expressing the Glu substitution were 3-fold more resistant. In each case, susceptibility to apoptosis correlated with caspase 3 activity.

All investigators agree that αB-crystallin can provide powerful antiapoptotic protection against multiple stresses. At least 2 pathways appear to account for this: (1) ischemia in cardiac myocytes or growth factor deprivation in differentiating skeletal myocytes activate αB-crystallin expression and promote translocation to myofilament and nuclear structures where the chaperone functions confer myofilament stabilization; and (2) the same stress conditions that promote translocation also favor increased association of αB-crystallin with other cellular proteins, including apoptosis regulators such as caspase 3 (p24). The translocation and binding activities may be related and both probably contribute to cytoprotection and suppression of apoptosis. Precisely how these activities are differentially regulated by serine phosphorylation is not clear. As shown by Morrison et al, Ser-59 phosphorylation is required to protect cardiac myocytes from ischemia, but the same phosphorylation appears to inhibit this function in differentiating skeletal myocytes. This is perplexing because caspase 3 was reported to be the target in both instances. Clearly the signaling cascades that are activated by ischemic stress are different from those that are active during myogenic differentiation and the outcome is determined by the net effect of multiple cross-reacting pathways. It may also be assumed that Ser-19 and Ser-45 phosphorylation will contribute to function under some circumstances. Two pre-
vious studies support a positive role of serine phosphorylation (see references 27 and 36 in Morrison et al).

The contribution of oligomerization to chaperone activity of αB-crystallin is also not resolved. Morrison et al suggest that αB-crystallin monomers may be the preferred chaperones whereas Ito et al and Martin et al conclude the opposite. It seems possible that the state of oligomerization of αB-crystallin is secondary to other factors that regulate function. Although further studies are required to explain these controversies, Morrison et al have made significant progress towards establishing a full signaling pathway for cytoprotection by αB-crystallin in ischemic cardiac myocytes.

References

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