Role of α-Crystallin B in Regulation of Stress Induced Cardiomyocyte Apoptosis

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Abstract: Cardiovascular disease is the leading cause of death worldwide. Recently emerging evidence suggests that cardiomyocyte apoptosis is one of the major pathogenic factors in heart diseases leading to heart failure. Cardiomyocytes undergo apoptosis in response to a wide variety of cellular stresses including protein folding stress at Endoplasmic reticulum (ER). Stressed myocytes elicit an adaptive response referred as Unfolded Protein Response (UPR) by inducing accumulation of heat shock proteins (HSPs) to mitigate the ER stress. HSPs act as molecular chaperons by assisting correct folding of the aggregated misfolded proteins in ER lumen. α-Crystallin B (CRYAB) is an abundant small HSP that confers protection to cardiomyocytes against various stress stimuli. Recent evidence indicates that CRYAB directly interacts with several components of ER stress and also mitochondrial apoptotic pathway. Based on currently available literature this mini review will focus on how CRYAB confers protection to stressed myocardium thereby emphasizing its function as antiapoptotic molecule. Understanding the interplay between CRYAB and the key components in the apoptotic signaling cascade mediated by ER and mitochondria will help in development of novel therapies for cardiac diseases.

Keywords: Apoptosis, cardiomyocyte, endoplasmic reticulum, heat shock proteins, mitochondria.

OVERVIEW

Cardiovascular disease and resulting heart failure are the leading causes of death worldwide [1]. Several studies in both human and animal models indicate progressive loss of terminally differentiated cardiomyocytes play a key role in the pathogenesis of a variety of cardiovascular diseases including heart failure [1, 2]. Therefore, counteracting apoptosis is considered to be a beneficial strategy in the treatment of heart failure [2].

Heat shock proteins (HSPs) were originally named because they were rapidly induced in response to elevated temperatures. But later, it has been shown that a wide variety of cellular stresses such as oxidative/osmotic or heavy metals stress were also equally capable of inducing HSPs [3, 4]. HSPs act as molecular chaperones to restore protein homeostasis and confer protection to cells during conditions of acute or chronic stress hence are most commonly known as stress proteins [5].

Based on their size and sequence homology, HSPs are grouped into several classes. Small HSPs have molecular masses ranging from 15-40 kDa. There exists striking similarities between vertebrate α-crystallins and small HSPs in terms of sequence, molecular diameter, secondary and tertiary structural features and heat-regulated transcriptional control of the promoter. α-Crystallins were originally found in abundance in eye where it had been shown to regulate transparency of the ocular lenses [6-9]. Among the two forms of closely related α-crystallins, α A and α B (CRYAB), latter is expressed at high levels in the heart [6]. Emerging evidence suggests that CRYAB acts as molecular chaperon and protects the heart against stress induced damage [9].

This minireview will focus on the activation of CRYAB and its interaction with various myocardial proteins particularly involved in intrinsic and Endoplasmic Reticulum (ER) stress pathways of apoptosis. Finally, it will point to CRYAB as an anti-apoptotic molecule providing protection to stressed myocytes and a rational target for the development of new drugs in various cardiac disease forms.

STRESS INDUCED ACTIVATION OF CRYAB IN CARDIOMYOCYTES:

It has been shown that heart in response to potentially harmful stresses such as ischemia, oxidative, osmotic or heat shock conditions activate p38 mitogen-activated protein kinase (MAPK) cascade [10]. This comes in agreement with the finding showing inhibition of MAPK/p38 pathway results in increased cardiomyocyte apoptosis and myocardial tissue damage in response to stress [10, 11]. Interestingly, recent reports have also indicated increases in CRYAB and phosphorylated CRYAB (p-CRYAB) levels following H2O2 exposure. Furthermore, CRYAB silencing results in increased apoptosis after exposure to H2O2 [12]. Also, CRYAB overexpression confers protection to cardiomyocytes against ischemia induced damage [13, 14]. A recent study reports cytoprotective role of p38 MAPK pathway during infarct
formation resulting in activation of CRYAB in male Wistar rats [15]. p38 MAPK which is activated, via MEKK3/MEKK6 pathways, stimulates the MAPK-activated protein kinase-2 (MAPKAPK-2) which in turn phosphorylates CRYAB on Ser-59 [10, 16, 17]. Although CRYAB is also phosphorylated on Ser-19 and 45 in response to stress but it has now been well accepted that Ser-59 is selectively responsible for mediating the cytoprotective actions in cardiac myocytes [10, 11, 18]. Also, significant reduction or increase in apoptosis compared to wild type is noted in primary cardiomyocytes in vitro expressing recombinant CRYAB mimicking or blocking phosphorylation at Ser-59, respectively [10] (Fig. 1).

Few reports also suggest ER-dependent induction of CRYAB as an adaptive response to ER stress [12, 19]. Accordingly, CRYAB is upregulated during ER stress whereas significant increase in apoptosis and decreased viability of cardiomyocyte is observed in CRYAB-silenced cells [12, 20]. One of the HSP70 family proteins that is constitutively expressed in the ER and assists in protein folding is BiP/glucose regulated protein 78 (GRP78/Bip) [21]. Under non-stress condition, BiP/GRP78, the master regulator of the UPR, interacts with other ER resident transmembrane proteins like PERK, ATF6 and IRE1. On activation of ER stress and upon accumulation of misfolded protein in the ER, it gets dissociated from such protein aggregation, to activate three independent pathways [21]. PERK-dependent pathway is activated following dimerization and autophosphorylation of its kinase domain, resulting in subsequent phosphorylation of eIF2α which finally help in attenuation of protein synthesis. During prolonged and unmitigated ER stress, this pathway uses ATF4 to induce expression of the proapoptotic proteins C/EBP homologous protein (CHOP), growth arrest and DNA damage 34 (GADD34) [22]. Similarly, IRE1 activation is responsible for splicing of XBP1 mRNA to yield XBP1s spliced variant. This potent transcription factor upregulates expression of both chaperons that enhance protein folding in ER and genes involved in ER associated degradation (ERAD) [21]. ATF6

![Schematic diagram showing induction of CRYAB in Cardiomyocytes under stress. Transcriptional activation of CRYAB by via IRE-1 and ATF6 pathways of UPR during ER stress and its phosphorylation by p38/MAPK pathway.](image)

**Fig. (1).** Schematic diagram showing induction of CRYAB in Cardiomyocytes under stress. Transcriptional activation of CRYAB by via IRE-1 and ATF6 pathways of UPR during ER stress and its phosphorylation by p38/MAPK pathway.
dependent pathway is activated when BiP/GRP78 and calreticulin (that remains bound to ATF6 under non-stress condition) dissociate from ATF6 which then translocates to the Golgi, where it undergoes cleavage by S1P and S2P proteases. This cleavage yields a cytoplasmic transcription factor (N-ATF6) that moves to the nucleus to activate UPR target genes like GRP78, GRP94 etc [21-23]. Stressed cells try to re-establish homeostasis through IRE1 and ATF-6 signaling, failing which the balance shifts in favour of PERK signaling induced apoptosis via ATF4-CHOP cascade [24]. CRYAB is reported to be one of the downstream effectors of two major UPR proteins IRE 1 and ATF6 [12, 19, 25, 26]. Accordingly, siRNA-mediated knockdown of IRE 1 and ATF6 have also shown to decrease CRYAB expression in the endothelial cells [25]. Taken together, these reports confirm the association of CRYAB with IRE1 and ATF-6 branches of UPR, which indicate its prosurvival role during ER stress (Fig. 1).

REGULATION OF ER STRESS INDUCED APOPTOTIC PATHWAY IN CARDIOMYOCYTES BY CRYAB

Cytoskeletal protein folding under normal condition is reported to be mediated by CRYAB [27]. A major effect of stress is unfolding and aggregation of intermediate filament network in cardiomyocytes that eventually lead to irreversible structural damage. Overexpression and Z disk localization of CRYAB during stress confirms its role in protection of myofilaments [28]. This is achieved by interaction of CRYAB with actin, α-actinin, desmin, titin, nebeulite in order to either stabilize the proper conformation of these various filaments or to prevent effectively their tendency to form aggregates at acidic pH [9, 18, 27, 28].

Various studies are indicative of the fact that phosphorylated CRYAB (p-CRYAB) targets cytosolic Caspase 3. p-CRYAB binds to and inhibits the proteolytic conversion of the inactive caspase-3 (p24) to its active forms (p20/p17/p12) [10]. Study on effect of mutations mimicking or blocking CRYAB phosphorylation at Ser-19, 45, and 59, it can be confirmed that phosphorylation at Ser-59 is necessary for caspase-3 inhibition in cardiac myocytes [10, 18]. Additionally, a study also pointed out the potential ability of active CRYAB to bind to caspase 3 even after its cleavage [12]. Another caspase activated by ER stress is Caspase 12. ER-resident caspase-12 might indirectly activate cytoplasmic caspase-3 or may also contribute to ER stress-induced apoptosis directly [29]. Both CRYAB and p-CRYAB interact with caspase 12 and prevent its activation thereby reducing ER stress-induced apoptosis in cardiomyocytes [12, 30] (Fig. 2).

Besides mediating apoptosis independently of mitochondria, ER (or sarcoplasmic reticulum in muscle cells) also contributes to mitochondrial death pathway [31]. The probable mechanism is suggested to be increase in intracellular Ca\(^{2+}\) level. In presence of apoptotic signals, increased ER Ca\(^{2+}\) positively regulates more Ca\(^{2+}\) release into cytosol [32]. Ca\(^{2+}\) overload triggers MPT pore opening in mitochondria resulting in cytochrome c release [33].

![Fig. (2). Regulation of Cardiomyocyte apoptosis by CRYAB.](image-url)
report shows α-crystallin pretreatment significantly reduces cytoplasmic Ca\(^{2+}\) concentration in the lymphocytes, hepatocytes and astrocytes of inflammation-induced mice [34]. Relatively little information is available on role of CRYAB in Ca\(^{2+}\) homeostasis in cardiomyocytes till date (Fig. 2).

**INTERACTION OF CRYAB WITH VARIOUS APOPTOTIC SIGNALING MOLECULES IN MITOCHONDRION**

Core molecular components of the mitochondrial permeability transition pore (MPTP) are the adenine nucleotide translocase (ANT), Cyclophilin D located in the inner mitochondrial membrane and VDAC1 and peripheral benzodiazepine receptor located in the outer mitochondrial membrane. In healthy cells, the inner mitochondrial membrane is relatively impermeable compared to the outer membrane. This helps to maintain both proton and osmotic gradient across the membranes. ANT is a ligand-specific translocase but in response to stress signals, ANT undergoes a conformational change and converts into a nonspecific pore allowing free passage of protons and water across the inner membrane. The influx of water down its osmotic gradient leads to swelling of the mitochondrial matrix and inner membrane resulting in rupturing of the outer mitochondrial membrane, releasing cytochrome c in the cytoplasm [31].

Members of the Bcl-2 family of apoptotic regulators are generally classified into two functional groups. First group comprises of Bcl-2 and Bcl-XL, possessing antiapoptotic ability while Bak, Bax and Bcl-XS form the second group that promotes apoptosis. Bcl-2 and Bcl-XL are localized on the membrane of mitochondria, ER and nucleus whereas a substantial fraction of Bax is found in the cytosol [35]. Upon stimulation by death signals Bax translocates from cytosol and inserts into the mitochondrial membrane where it can either directly induce the release of cytochrome c by forming protein or lipid pores or may also form heteroligomers with Bak/VDAC1 to form permeability transition pores for pro-apoptotic factor efflux. Normally, VDAC1 prevents cytochrome c release but the formation of hetero-oligomers composed of VDAC1 and Bax in the outer mitochondrial membrane allow cytochrome c efflux from the mitochondrion [36]. This is supported by reports which have shown addition of Bax to liposomes containing VDAC changes its property of voltage-dependent channel to voltage independent nonselective pores [36, 37]. Like Bax, endogenous Bcl-XS is also distributed in the cytosol, but is translocated to the mitochondrial membrane upon overexpression [35].

Recent studies demonstrate that α-crystallin executes blockage in the mitochondrial pathway of apoptosis at various steps [35]. Coimmunoprecipitation assays reveal increased level of interaction of Bak and Bcl-XS with CRYAB compared to CRYAB mutant R120G [35]. R120G mutation is an arginine to glycine substitution at residue 120 in CRYAB and is linked to decreased protection against stress induced apoptosis [35, 38]. One report has shown after staurosporine stimulation CRYAB sequesters Bak and Bel-XS from the cytosol and prevents their translocation into mitochondria more effectively compared to R120G mutants [35]. It has also been shown that p-CRYAB (Ser59) translocates to the stressed mitochondrial surface in response to the unfolding of mitochondrial proteins where it binds to and modulates the function of MPT pore openings [26]. Additionally, by interacting with VDAC1 channel, CRYAB and/or p-CRYAB stabilize the mitochondrial membrane preventing cytochrome c release from mitochondrial intermembrane space [19, 12] (Fig. 2).

**THERAPEUTIC PROSPECTS OF CRYAB**

Loss of cardiomyocytes by apoptosis due to prolonged oxidative stress often leads to heart failure [18]. Since cardiomyocytes have a very limited proliferative capacity, it is always desirable to prevent myocyte apoptosis under severe stress. Several intrinsic factors which include antioxidants, antiapoptotic factors such as Bcl-2 proteins, and endogenous caspase inhibitors works to attenuate oxidative stress induced damage in cardiomyocytes [40]. These antiapoptotic molecules have emerged as potential therapeutic targets for prevention of myocyte apoptosis [40]. Furthermore, small HSP’s are already in clinical trials for the treatment of neurodegenerative diseases [41]. Therapeutic role for CRYAB in stroke is shown in one report [42]. Another study demonstrates that CRYAB therapy improves outcome of both acute and progressive phases of autoimmune encephalomyelitis (EAE) [43]. Although many studies have pointed out critical structural as well as protective functions of α-crystallins but much less data is available on the therapeutic effects of CRYAB in heart. One report has clearly shown exogenous administration of CRYAB significantly improves impaired cardiac function in mice after I/R injury in vivo [44]. Thus, considering strong antiapoptotic and chaperoning properties of CRYAB, further studies are required to establish it as an attractive target for future therapeutic options for cardiac diseases [18, 19].

**CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflict of interest.

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CRYAB</td>
<td>α-Crystallin B</td>
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<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
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<td>HSPs</td>
<td>Heat shock proteins</td>
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<tr>
<td>UPR</td>
<td>Unfolded Protein Response</td>
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<tr>
<td>GRP78/Bip</td>
<td>BiP/glucose regulated protein (GRP)78</td>
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<tr>
<td>ERAD</td>
<td>ER-associated degradation pathway</td>
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<tr>
<td>eIF2 α</td>
<td>Eukaryotic initiation factor 2</td>
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ATF6 = Activating transcription factor-6
Mit = Mitochondria

REFERENCES


