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Chapter 2

The protective role of small heat shock proteins in cardiac diseases: key role in atrial fibrillation

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Abstract

Atrial fibrillation (AF) is the most common tachyarrhythmia which is associated with increased morbidity and mortality. AF usually progresses from a self-terminating paroxysmal to persistent disease. It has been recognized that AF progression is driven by structural remodeling of cardiomyocytes which results in electrical and contractile dysfunction of the atria. We recently showed that structural remodeling is rooted in derailment of proteostasis, i.e. homeostasis of protein production, function and degradation. Since heat shock proteins (HSPs) play an important role in maintaining a healthy proteostasis, the role of HSPs was investigated in AF. It was found that especially small heat shock proteins (HSPBs) levels get exhausted in atrial tissue of patients with persistent AF and that genetic or pharmacological induction of HSPB protects against cardiomyocyte remodeling in experimental models for AF. In this review, we provide an overview of HSPBs as a potential therapeutic target for normalizing proteostasis and suppressing the substrates for AF progression in experimental and clinical AF and discuss HSP activators as a promising therapy to prevent AF onset and progression.

AF progression by structural remodeling

Atrial fibrillation (AF) is an age-related tachyarrhythmia in both left and right atria, which can be caused by underlying (heart) conditions, such as valvular heart disease, congestive heart disease, ischemic cardiomyopathy, obesity, diabetes mellitus and hypertension [1, 2]. The goal of AF therapy is, ideally, to abolish AF episodes and to restore normal sinus rhythm. Unfortunately, treatment of AF remains difficult, which is caused by the persistent and progressive nature of this arrhythmia. There are strong indications that remodeling of the structure of atrial cardiomyocytes underlies electrophysiological and contractile dysfunction and AF perpetuation [3]. Structural remodeling includes degradation of sarcomeres (the smallest contractile units of the cardiomyocytes), namely myolysis, by proteases such as calpain [4, 5] and disruption of the microtubule network [6], which result in impaired electrical coupling and functional recovery to sinus rhythm after pharmacological and electrical cardioversion [7-9]. Importantly, structural changes are already presented when a patient enters the clinic for the first time with an episode of AF. Since the current available therapy is directed at alleviation of electrophysiological changes (rhythm control), it has limited effect on patient's outcome. Therapeutic approaches that counteract the pathways conveying AF-induced structural remodeling may offer superior therapeutic perspectives. Recent research findings indicate that derailment of proteostasis, i.e. the homeostasis of protein production, function and degradation, constitutes an important factor for the induction and progression of AF. In addition, it was observed that especially small heat shock proteins (HSPBs) convey protective effects against derailment of proteostasis and thereby attenuate structural remodeling, AF onset and progression.

Proteostasis and role for HSPs

It has been recognized that a proper function of cells and organisms depends critically on the maintenance of a healthy proteostasis [10, 11]. Proteostasis is particularly important in long-lived post-

mitotic cardiomyocytes, since they display limited regenerative capacity. Proteostasis involves controlling the concentration, conformation, binding interaction, kinetics and location of individual proteins. Derailment of cellular proteostasis results in many systemic diseases, including cardiovascular disorders [10]. Cells respond to a loss of proteostatic control by inducing a heat shock response (HSR), upon which HSPs are expressed. Interestingly, in the heart, numerous HSPs are already expressed at high levels, especially the family of small HSPs members: HSPB1, HSPB5, HSPB6, HSPB7 and HSPB8. These HSPBs are considered to constitute the cell's first line of defense against proteostasis derailment by stabilizing the sarcomeres [5, 12]. In general, HSPs act as molecular chaperones to facilitate protein folding, localization, degradation and function, thereby maintaining proteostasis and preventing various forms of cardiomyocyte damage [13]. Indeed, HSPs were found to play a protective role in various cardiovascular diseases, including AF. Two studies reported induced expression of mitochondrial HSPs, HSPD1, HSPE1 and mortalin (HSPA9B) in atrial tissue of patients with AF. These HSPs may play a protective role by maintaining mitochondrial integrity and capacity for ATP generation [14, 15]. Unfortunately, no mechanistic studies have been performed to conclusively address their function. Other studies revealed induced HSPA1A expression in atrial tissue of patients undergoing cardiac surgery. Higher HSPA1A expression correlated with lower incidence of post-operative AF, suggesting a cardio-protective role for HSPA1A [16, 17]. A key role for HSPB members in the protection against AF onset and progression was identified in several studies [12, 18-20]. Interestingly, it was found that overexpression of HSPB1 protects against contractile dysfunction by conservation of the cardiomyocyte structure in the tachypaced HL-1 cardiomyocyte model for AF and in clinical AF [12], suggesting HSPB1 to represent a druggable target in AF.

Key role for HSPB members in the prevention of cardiac diseases

HSPB members

The family of HSPBs consists of at least 10 members and they are expressed in various human tissues

[21] (Table 1). HSPB members are defined by a conserved C-terminal domain of approximately 90 amino acids (the α -crystallin domain) flanked by a variable length N-terminal arm and a more conserved C-terminal extension [22]. Some HSPB members, including HSPB1, HSPB5 and HSPB8, are thought to assemble into homo- and/or heterogeneous oligomeric complexes, which dissociate into smaller multimers upon stress. Another important characteristic is that various HSPB members can be phosphorylated, which changes their activity and oligomeric state [23].

Table 1. Characteristics of HSPB members

Gene name	Protein name	Alternative name	Molecular weight (kDa)	Expression in heart	References	Other tissue Expression
HSPB1	HSPB1	DmHSP23, HSP25, HSP27	22.783	+	(Golenhofen et al., 2004) (Vos et al., 2009)	Uterus, skin, Platelets, brain, Kidney, some tumor cells
HSPB2	HSPB2	MKBP	20.233	+	(Sugiyama et al., 2000) (Vos et al., 2009) (Ishiwata et al., 2012)	Skeletal muscle
HSPB3	HSPB3	HSP127	16.966	-	-	Skeletal muscle
HSPB4	HSPB4	α A-Crystallin, CRYAA, CRYA1	19.909	-	-	Lens of eye, spleen
HSPB5	HSPB5	α B-Crystallin, CRYAB, CRYA2	20.159	+++	(Bennardini et al., 1992) (Vos et al., 2009) (Cubedo et al., 2016)	Lens of eye, vascular wall muscle, lung, Kidney, brain, some tumor cells
HSPB6	HSPB6	HSP20	17.136	++	(Verschuure et al., 2003) (Golenhofen et al., 2004) (Fan et al., 2005) (Vos et al., 2009)	Skeletal muscle, stomach, liver Lung, kidney, platelet
HSPB7	HSPB7	CvHSP	18.611	+++	(Fan and Kranias, 2011) (Krief et al., 1999) (Verschuure et al., 2003) (Golenhofen et al., 2004) (Vos et al., 2009)	Skeletal muscle
HSPB8	HSPB8	HSP22, H11 kinase (H11K)	21.604	+++	(Verschuure et al., 2003) Vos et al., 2009	Skeletal muscle, stomach, liver Lung, kidney, brain
HSPB9	HSPB9	FLJ27437	17.486	-	-	Testis
HSPB10	HSPB10	ODF1	28.366	-	-	Testis

HSPBs have at least three, not mutually exclusive, biochemical functions within the proteostasis regulation. Firstly, both *in vitro* and *in vivo* findings suggested that some HSPB members act as ATP-independent chaperones by preventing irreversible protein aggregation [24-26]. Oligomeric dynamics seem to be crucial for such HSPB activities [27]. HSPB clients may also be processed (renaturation or degradation) through cooperation with ATP-dependent chaperones [28, 29]. As such, stress-induced protein damage, which may also occur in AF, can be prevented. Secondly, several HSPB members are associated with cytoskeletal proteins in a phosphorylation-dependent manner [30-32]. This results in stabilization of cytoskeletal structures and increased resistance to stress situations, including AF. Finally, HSPB members are found to inhibit the activation of proteases and as such may prevent the activation of calpain, which was found to become activated in clinical AF [4, 18].

Relevant HSPB family members for heart function: functional similarities and divergence

Various HSPB members are expressed at high levels in the heart [32, 33] (Table 1). One of these members is HSPB1. HSPB1 can exist as high or low molecular weight structures. Under normal, non-stressed conditions, a high molecular weight structure is the most predominant form. During proteotoxic stress, its level decreases whereas the level of phosphorylated low molecular weight structures increases [23]. In addition to the role of HSPB1 in assisting in refolding and/or targeting denatured proteins, another well-studied role of HSPB1 is its ability to interact with several cytoskeletal proteins, including actin, intermediate filaments and microtubules [30, 34]. In heart tissue, HSPB1 is found associated with sarcomeres and thereby was found to be cardio-protective [12].

HSPB2 associates specifically with dystrophy myotonic protein kinase (DMPK) and therefore is called a DMPK-binding protein, indicating its importance in muscle maintenance [35, 36]. It is highly expressed in heart and skeletal muscle and was found to have protective effects against cardiac diseases, such as cardiac hypertrophy and ischemia heart diseases [37-39]. Also, HSPB2 was found to be associated with the outer membrane of mitochondria, thereby regulating the mitochondria permeability transition and

calcium uptake in mitochondria. Overexpression of HSPB2 was found to conserve ATP synthesis in mice with ischemia/reperfusion injury [39]. Mice with specific knock out of HSPB2 show, upon ischemic stress, reduced mitochondria respiration rates and ATP production as well as suppression in expression of several metabolic and mitochondrial regulators [38]. These findings imply that HSPB2 is cardio-protective via maintenance of mitochondrial function and metabolic activity during cardiac stress. This role has been confirmed in a study utilizing a double knockout of HSPB2 and HSPB5. Here, inhibition of mitochondrial calcium signaling and, consequently, a reduction in ATP synthesis were observed during ischemia/reperfusion [36]. Findings from the study of Golenhofen et al. imply that the increased calcium in the cytosol, due to knock out of HSPB2, may modify the calcium sensitivity of myofibrils, contributing to malfunction of cardiac contractility [40]. Interestingly, mice overexpressing cardiac HSPB2 revealed lower levels of cardiac biomarker troponin I in the blood after ischemia/reperfusion injury, indicating that troponin I levels in heart tissue are conserved, thereby preserving contractile function of the heart [41].

HSPB3 and HSPB4 are not expressed in heart [21], whereas HSPB5 co-localizes on the I-band and M-line region of sarcomeres in cardiomyocytes [42]. HSPB5 is known to bind and stabilize intermediate filaments, actin microfilaments and sarcomeric proteins, including actin, desmin and titin [43-45]. Like HSPB1, HSPB5 also plays an important role in stabilization of the cytoskeleton as it is expressed together with HSPB1 to associate with sarcomeric proteins [34]. Mutations in HSPB5 are associated with a broad variety of neurological, cardiac and muscular disorders. The R120G mutation results in an irregular protein structure and defective chaperone-like function [46], which may accelerate the accumulation of desmin aggregation, thereby leading to desmin-related myopathy and also early onset of cardiomyopathy [34, 47].

HSPB6 is abundantly expressed in skeletal muscle and heart in two complex formations: 43 kDa dimers and 470 kDa multimers. HSPB6 binds to itself and other HSPBs (HSPB1, HSPB5 and HSPB8) [48]. Recently, HSPB6 overexpression was found to result in enhanced cardiac function by interacting with

protein phosphatase 1, thereby inducing Ca^{2+} cycling and sarcoplasmic reticulum Ca^{2+} load [49]. In addition, in HSPB6 transgenic rat ventricular cardiomyocytes, HSPB6 increases the phosphorylation at specific sites of the calcium regulatory protein phospholamban, via inhibition of protein phosphatase 1. As such, HSPB6 promotes the Ca^{2+} cycling in the sarcoplasmic reticulum and enhances the contractile function of the cardiomyocyte [49]. Moreover, another study described HSPB6 to reduce the myocardial infarcted area, thereby conserving the heart integrity in mice with ischemia/reperfusion injury [50]. Besides, the phosphorylation on of HSPB6 at serine 16 was found to be required for attenuating ischemia/reperfusion-induced cell injury in mice, as the non-phosphorylatable HSPB6 induced apoptosis and necrosis, suppressed the autophagy activity and subsequently depressed the cardiac functional recovery during ischemia and reperfusion [51].

HSPB7 is expressed in heart and skeletal muscle. In aged muscle, it was shown that both HSPB5 and HSPB7 expression are dramatically increased [52]. HSPB7 upregulation is also found in the muscular dystrophy-affected diaphragm, indicating that HSPB7 levels are induced under stress conditions. Furthermore, HSPB7 protects cells from protein aggregation, likely by facilitating cargo delivery to autophagosomes [53]. Interestingly, HSPB4, HSPB6, or HSPB7 could not enhance the cellular capacity to chaperone heat-denatured luciferase, in contrast to HSPB1, indicating further functional differentiation of the HSPB members [53, 54]. In addition, co-localization of HSPB7 on myofibrils in cardiomyocytes is observed [32], suggesting a protective role via conservation of the sarcomeric structure.

HSPB8 is strongly expressed in striated and smooth muscles, brain and keratinocytes [23]. Like HSPB1 and HSPB5, HSPB8 can also be phosphorylated *in vitro*. In contrast to HSPB1 and HSPB5, phosphorylation of HSPB8 only marginally affects its tertiary and quaternary structure. Both wild-type and phosphorylated HSPB8 exist as low-molecular mass oligomers. Unlike HSPB1 and HSPB5, where phosphorylation increases chaperone activity and reduce oligomeric size, phosphorylation of HSPB8 results in larger oligomeric structures and severely lowered chaperone activity [55]. In *in vitro*

experiments, HSPB8 interacts with several proteins and forms stoichiometric complexes with Bag3, a co-factor of HSPA1A [56]. The Bag3/HSPB8 complex was found to induce both translational arrest and autophagy, which may be beneficial in response to irreparable protein damage [24, 57]. In addition, HSPB8 is cardio-protective in experimental models of myocardial ischemia. Overexpression of HSPB8 promotes cardiomyocyte survival after ischemia in mice [58] and attenuates the myocardial damage and contractile dysfunction in pig [59], whereas depletion of HSPB8 in mice with pressure overload contributes to cardiac dysfunction and accelerates transition to heart failure [60]. Furthermore, studies show HSPB8 to conserve mitochondrial function and energy production, thereby attenuating oxidative stress in infarcted hearts [61]. In contrast to these beneficial effects of HSPB8 on cardiomyocyte function, overexpression of HSPB8 was also found to induce cardiac hypertrophy both in *in vitro* and *in vivo* model systems and re-expression of the cardiac fetal gene program and provoked cell growth pathways as well as proteasome activities [62, 63]. Therefore, the function of HSPB8 seems two-edged in heart diseases: HSPB8 reveals beneficial effects on myocardial ischemia by conserving the mitochondrial function and energy production, and HSPB8 is a mediator of cardiac hypertrophy and thereby results in heart failure.

Interestingly, various HSPB family members have common functions by translocating from cytoplasm to specific sarcomeric proteins upon different forms of stress (Table 2). During aging, HSPB1 was found to translocate from the cytoplasm of ventricular cardiomyocytes to the sarcomeric actin in the Z-line [64]. Under acidic stress, HSPB1 translocates from the cytosol to the unfolded Ig domain of titin on the I-band, to prevent its aggregation resulting in maintenance of titin function. Moreover, HSPB1 is co-localized with the titin spring in the elastic I-band region in dilated cardiomyopathy patients, while HSPB1 is mainly expressed in the cytoplasm of cardiomyocytes in the healthy heart. In addition, phosphomimicking HSPB1 mutants did not alter its binding to titin, compared to wild-type HSPB1 [65], indicating that binding of HSPB1 to titin is phosphorylation dependent. In the ischemic heart, co-localization of HSPB2 at the Z-line of sarcomeres was enhanced [66]. Moreover, in *in vivo* studies, HSPB5 was found to be soluble in the cytosol of cardiomyocytes under normal control conditions,

while, in the ischemic heart, HSPB5 was phosphorylated, resulting in the transition of soluble HSPB5 to insoluble fractions and translocation from the cytosol to myofibrils. Furthermore, it was demonstrated in a pig model that HSPB5 strongly binds to titin after translocation to myofibrils. The chaperone activity is required to prevent the unfolding and irreversible derailment of myofibrils [67-69]. HSPB6 associates with HSPB5 and localizes in the distinct transverse bands in the similar pattern as sarcomeric actin, indicating that it probably modulates the contractile dynamics in cardiac myocytes through associating with sarcomeric actin [48].

Protective role of HSPB members in AF

So far, various HSPB members are found to be protective against AF (Table 3). In atrial tissue of patients with AF, HSPB1 localizes at sarcomeres [12]. Furthermore, HSPB1 overexpression prevents the degradation of sarcomeric proteins in tachypaced HL-1 cardiomyocytes [12], indicating a prominent role for HSPB1 in conservation of the sarcomeric structure and function. Next to HSPB1, also HSPB6, HSPB7 and HSPB8 display protective effects against cardiomyocyte remodeling in tachypaced HL-1 cardiomyocytes [19]. As several HSPB members can form hetero-oligomeric complexes with each other, the protective effect of the various members may be due to the supportive oligomeric structures with HSPB1 [23, 27]. As downregulation of endogenous HSPB1 did not impair the protective effects of HSPB6, HSPB7 and HSPB8 in tachypaced HL-1 cardiomyocytes, their effects seem independent of endogenous HSPB1 [19]. Interestingly, all the protective HSPB members were able to reduce the formation of F-actin stress fibers, supporting the view that actin is the key target of the HSPB members in AF. Yet, the mode of action in preventing F-actin stress bundle formation of the four protective HSPB members seem to differ. Whereas HSPB8 interferes with the upstream tachypacing-induced RhoA GTPase activation, HSPB1, HSPB6 and HSPB7 do not. Rather, HSPB1, HSPB6 and HSPB7 bind to actin and directly inhibit G- to F-actin polymerization and/or stimulate depolymerization, indicating a protective role against tachycardia remodeling downstream of RhoA GTPase activation [19].

Table 2. HSPB binding to sarcomere structural proteins

Protein name	Experimental model	Myofibrillar protein targets	Phosphorylation dependency	Stress conditions	Reference
HSPB1	Ventricular cardiomyocytes	Sarcomeric actin	-	Aging	(Lutsch et al., 1997)
	Cardiomyocytes	Titin	Independent	Acidic stress; Dilated cardiomyopathy	(Kotter et al., 2014)
	Soleus muscle fibers	Z disc	Dependent	Hind limb reloading	(Kawano et al.)
	Biceps brachii muscle	Z disc	-	High force eccentric exercise	(Paulsen et al., 2009)
HSPB2	Cardiomyocytes	Z line	-	Ischemia	(Yoshida et al., 1999)
	Cardiomyocytes	Titin	Dependent	Ischemia	(Golenhofen et al., 2002; Golenhofen et al., 1999; Golenhofen et al., 1998)
HSPB6	Cardiomyocytes	Sarcomeric actin1	-	Normal condition	(Pipkin et al., 2003)
HSPB7	Skeletal muscle	Dimerized filamin C on Z line1	-	HSPB7 KO induced myopathy	(Juo et al., 2016)
HSPB8	-	-	-	-	-

*HSPB3 and HSPB4 are not expressed in heart; - is unknown; KO is knockout

Table 3. Summary of roles of several HSPBs in AF

HSPB	OE	Experimental model	Consequences	References	Other studied cardiac diseases
HSPB1	+	HL-1 cardiomyocytes	↑CaT	(Brundel, Henning, et al., 2006)	Ischemia/Reperfusion
			↑CS	(Brundel, Hiroshita-Takeshita, et al., 2006)	
			↓myolysis	(Ke et al., 2011)	
			Colocalization with myofibrils and actin filament		
			↑Heart contraction rate	(Zhang et al., 2011)	
			↓Duration of arrhythmia episode		
			↑Heart wall shortening		
			↓Ultrastructural damage		
			↓Calpain activity		
			↓F-actin stress fiber		
HSPB6	+	HL-1 cardiomyocytes	↑CaT	(Ke et al., 2011)	Ischemia/Reperfusion
			↓F-actin stress fiber		
HSPB7	+	HL-1 cardiomyocytes	↑CaT;	(Ke et al., 2011)	Ischemia/Reperfusion
			↓F-actin stress fiber		
HSPB8	+	HL-1 cardiomyocytes	↑CaT	(Ke et al., 2011)	Ischemia/Reperfusion
			↓RhoA GTPase activity		
			↓F-actin stress fiber		

Next to the protective effects on F-actin stress bundle formation, HSPB1 conserves the calcium handling. HSPB1 overexpression protects against loss in Ca^{2+} transients and cell shortening in tachypaced HL-1 cardiomyocytes and this protective effect is phosphorylation-dependent, as a non-phosphorylatable HSPB1 mutant did not show an effect [12]. In addition, the protective effect on the calcium handling may involve the direct modulation of ion channel function or modulation of specific kinases, resulting in the conservation of ion currents, including the L-type Ca^{2+} current [70]. Previously, HSPs were found to regulate ion channel function in heart and brain [71-74]. Some HSPs were found to interact directly with ion channels, such as HSPB5 with Na^+ channels [71] and HSPA1A with cardiac K^+ channel HERG [72] and voltage-gated Ca^{2+} channels [73], suggesting a possible role for HSPBs in AF attenuation by interacting with ion channels.

HSPBs may also protect against AF by affecting signaling cascades that are activated by AF. HSPB1 associates with several kinases, such as $\text{I}\kappa\text{B}$ kinase and c-Jun N-terminal kinase (JNK), thereby suppressing activation of the transcription factor NF- κB [75, 76]. Interestingly, these kinases were reported to be modulated during AF [77-79].

Finally, HSPBs may prevent cardiomyocyte remodeling via inhibition of proteases, such as calpain. In tachypaced *Drosophila* overexpressing dmHSP23, likely the functional orthologue of human HSPB1, prevented calpain activation, myolysis and heart wall contractile dysfunction [18]. This finding is in line with a study showing that HSPB1 prevents ischemia/reperfusion-induced degradation of the contractile proteins cardiac troponin I and troponin T by interacting with the COOH-terminus and NH_2 -terminus, respectively. This interaction prevented calpain from cleaving cardiac troponin I and T and resulted in conservation of the contractile function in ventricular cardiomyocytes [80]. Also, HSPB1 colocalizes with cardiac troponin T in ventricular cardiomyocytes after morphine withdrawal, thereby preventing its degradation by calpain and maintaining myocardial function [81]. These findings together imply that HSPB1 binds to contractile proteins, thereby sequestering the proteolytic cleavage regions from calpain (Figure 1).

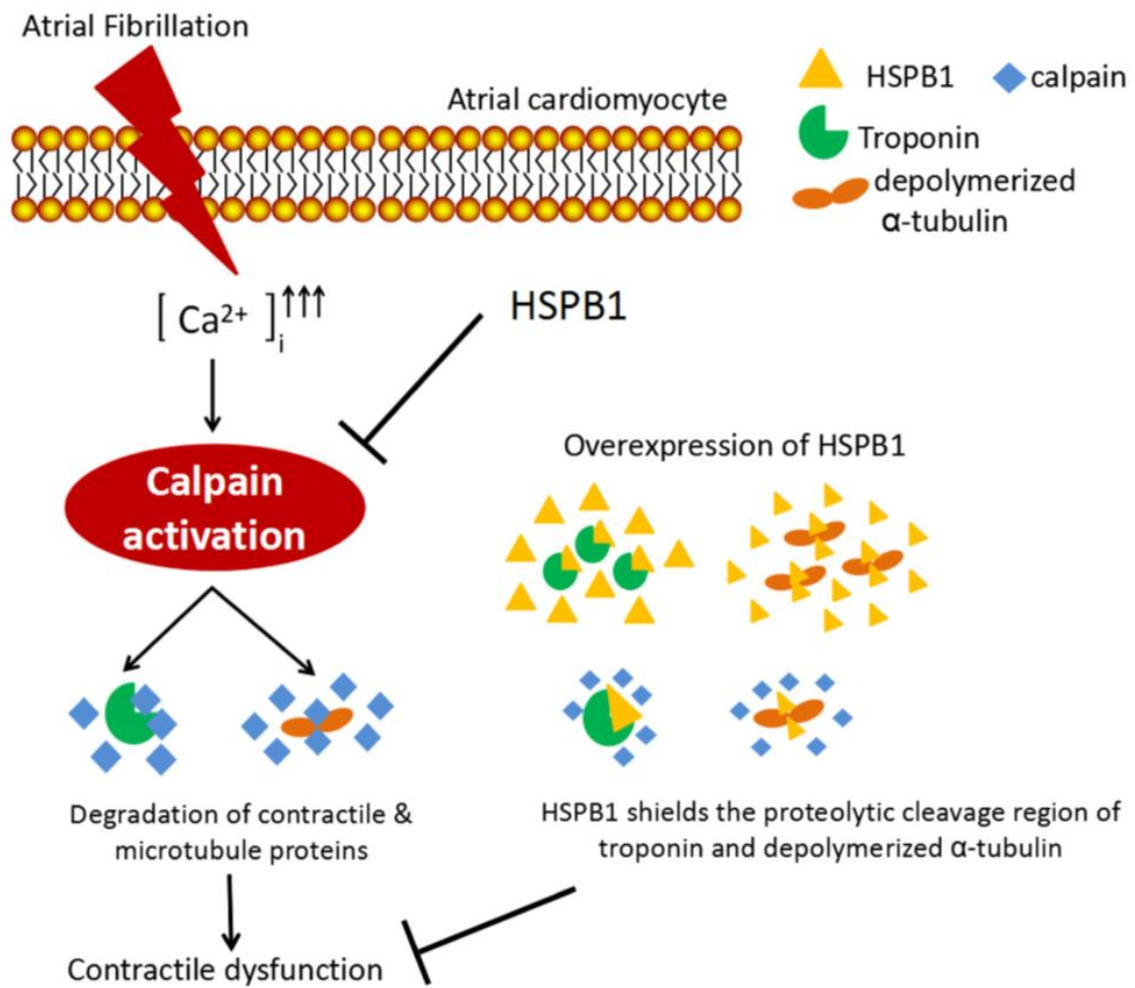


Figure 1. AF induces a calcium overload in cardiomyocytes, which activates calcium-dependent neutral protease calpain. Calpain degrades contractile proteins and the microtubule network resulting in structural remodeling, contractile dysfunction of cardiomyocytes and AF progression. Elevated HSPB1 is found to inhibit calpain activity in tachypaced *Drosophila*. In addition, HSPB1 prevents degradation of cardiac troponins and may protect against depolymerization of α -tubulin by sequestering the proteolytic cleavage sites from calpain.

HSPB in patients with AF

The experimental findings on the protective role of HSPB members in AF are in line with observations in paroxysmal and persistent AF patients. In patients with AF, an inverse correlation between the amount of HSPB1 expression and the level of myolysis and the duration of persistent AF is found [12]. Patients with longstanding persistent AF reveal reduced levels of HSPB1 compared to paroxysmal AF patients, suggesting that HSPB1 induction may represent a therapeutic target in longstanding

persistent AF patients. Furthermore, HSPB members are found to represent a biomarker for AF onset and progression and may also predict the clinical outcome after interventions. A recent study showed that the serum HSPB1 levels of patients who received catheter ablation predict AF recurrences. Patients with high levels of HSPB1 in serum show improved maintenance rate of sinus rhythm [82]. Because of the pleiotropic cardio-protective effects of HSPB1 on AF substrate formation, HSP inducers currently represent a class of drugs with promising therapeutic potential in clinical AF.

Therapeutic application of HSP induction in experimental and clinical AF

Previous research has demonstrated that the (genetic) induction of HSPB members provides prevention effects on tachycardia-induced structural remodeling and contractile dysfunction. A drug often used to boost HSP expression is geranylgeranylacetone (GGA) [2]. GGA is originally used as an anti-ulcer agent and is a non-toxic acyclic isoprenoid compound with a retinoid skeleton that induces HSP synthesis in various tissues, including gastric mucosa, intestine, liver, heart, retina and the central nervous system [83, 84]. GGA induces HSP expression probably via the activation of the heat shock transcription factor 1 [5]. The protective effect of GGA-induced HSP expression on structural remodeling has been observed in experimental models of AF, suggesting that the induction of HSPs by GGA may have a potential value for clinical AF [5, 12]. In tachypaced *Drosophila*, GGA treatment protects against contractile dysfunction of the heart wall and structural remodeling [18]. Furthermore, in canine models for (acute) atrial ischemia-related AF and tachypacing-induced AF promotion, GGA treatment reveals protective effects against cardiomyocyte remodeling and consequently occurrence and recurrence of AF after cardioversion [12, 85].

In addition to the pharmacological induction of HSPB, exercise is also found to induce HSPB levels and subsequently reveal cardio-protective effects. Various studies show that gene and protein levels of HSPB1 and HSPB6 are elevated after physical exercise in rat and mouse models. Interestingly, in these

studies HSPB1 and HSPB6 were phosphorylated, resulting in stabilization of myofilaments, restoration of disrupted contractile proteins and consequently improved the contractile function of the heart [86-91]. Therefore, physical exercise may represent a promising therapeutic therapy to ameliorate the cardiac function and quality of life in patients with AF and maintain normal sinus rhythm after cardioversion, via induction of HSPB levels.

Conclusion

Various HSPB members conserve a healthy proteostasis of cardiomyocytes and thereby prevent AF onset and progression. Their mode of action is via the stabilization of the cardiomyocyte structure, thus conserving the contractile and electrophysiological function of the atria. Since compounds, such as GGA, and exercise are found to induce HSPB expression, these may represent promising novel therapeutic strategies to prevent AF onset and progression.

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