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Targeting proteostasis in atrial fibrillation

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Chapter 2

Loss of proteostatic control as a substrate for Atrial Fibrillation; a novel target for upstream therapy by Heat Shock Proteins

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Abstract

Atrial Fibrillation (AF) is the most common, sustained clinical tachyarrhythmia associated with significant morbidity and mortality. AF is a persistent condition with progressive structural remodeling of the atrial cardiomyocytes due to the AF itself, resulting in cellular changes commonly observed in ageing and in other heart diseases.

While rhythm control by electrocardioversion or drug treatment is the treatment of choice in symptomatic AF patients, its efficacy is still limited. Current research is directed at preventing first-onset AF by limiting the development of substrates underlying AF progression and resembles mechanism-based therapy. Upstream therapy refers to the use of non-ion channel anti-arrhythmic drugs that modify the atrial substrate- or target-specific mechanisms of AF, with the ultimate aim to prevent the occurrence (primary prevention) or recurrence of the arrhythmia following (spontaneous) conversion (secondary prevention).

Heat shock proteins (HSPs) are molecular chaperones and comprise a large family of proteins involved in the protection against various forms of cellular stress. Their classical function is the conservation of proteostasis via prevention of toxic protein aggregation by binding to (partially) unfolded proteins. Our recent data reveal that HSPs prevent electrical, contractile and structural remodeling of cardiomyocytes, thus attenuating the AF substrate in cellular, and animal experimental models. Furthermore, studies in humans suggest a protective role for HSPs against the progression from paroxysmal AF to persistent AF and in recurrence of AF.

In this review, we discuss upregulation of the heat shock response system as a novel target for upstream therapy to prevent derailment of proteostasis and consequently progression and recurrence of AF.

1. Management of atrial fibrillation by upstream therapy

Atrial fibrillation (AF) is the most common clinical tachyarrhythmia which significantly contributes to cardiovascular morbidity and mortality, mainly through stroke and heart failure. The incidence of AF is escalating due to the increased prevalence of risk factors constituting a substrate for AF, such as obesity¹, metabolic syndrome² and increasing age.³ In addition to the increased first-onset of AF, also the progression of the arrhythmia poses problems, as the longer AF persists the less effective pharmacological and electrical cardioversion therapies are.⁴ In patients with symptomatic AF, rhythm control is the treatment of choice.⁵ However, the effective reversal to sinus rhythm is still inadequate. Therefore, recent research focuses on the identification of mechanisms underlying AF substrate induction and maintenance, which have led to several novel upstream therapeutic approaches, including angiotensin-converting enzyme inhibitors, aldosterone antagonists, statins and polyunsaturated fatty acids.⁶ Upstream therapy refers to the use of non-ion channel anti-arrhythmic drugs that modify the atrial substrate- or target-specific mechanisms of AF with the ultimate aim to prevent the occurrence (primary prevention) or recurrence (secondary prevention) of the arrhythmia.^{7,8}

It has been recognized that electrical and structural remodeling of cardiomyocytes create a substrate for AF.⁹ Nevertheless, the exact molecular mechanisms that underlie cardiomyocyte remodeling and AF progression are as yet unidentified. We recently obtained evidence that derailment of proteostasis (i.e. the homeostasis of protein production, breakdown and function) constitutes an important substrate for induction and progression of AF.¹⁰⁻¹² 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.¹³ Cells respond to a loss of proteostatic control by inducing the heat shock response (HSR) resulting in the expression of heat shock proteins (HSPs) that facilitate protein folding and function.¹⁴ Consequently, an emerging target candidate for upstream therapy of AF is the upregulation of HSPs. Indeed, HSP induction alleviates the occurrence and recurrence of AF in various experimental model systems for AF.^{12,15-17} Furthermore, studies in humans suggest a protective role for HSPs against progression from paroxysmal AF to persistent AF¹⁵ and the restoration of sinus rhythm in patients with persistent AF (secondary prevention).¹⁸

Here we discuss the concept that derailment of cardiomyocyte proteostasis constitutes an important aspect of the substrate for AF. In addition we examine the evidence for induction of the HSR system as a novel target for upstream therapy to prevent the occurrence and the recurrence of AF and address its possible modes of action.

2. Mechanisms underlying AF initiation and progression; derailment of proteostasis

Although the exact molecular mechanism(s) underlying AF initiation, maintenance and progression has not yet been completely elucidated, an important recognition was that AF induction required a suitable substrate as well as a trigger that acts on the substrate.⁹ Various clinical conditions, e.g. several heart diseases, hypertension and cardiac surgery, are risk factors for the first-onset of AF, as they create a substrate(s) and/or trigger(s) for the initiation of AF (Figure 1)¹⁹⁻²¹. Key AF promoting factors have been identified, including inflammation, oxidative stress, active Rho-GTPase, fibrosis and atrial muscle bundle dissociation^{22,23}, which induce loss of proteostatic control, creating a substrate for AF. Subsequent triggers will act on the substrate and will induce AF.²⁴⁻²⁷

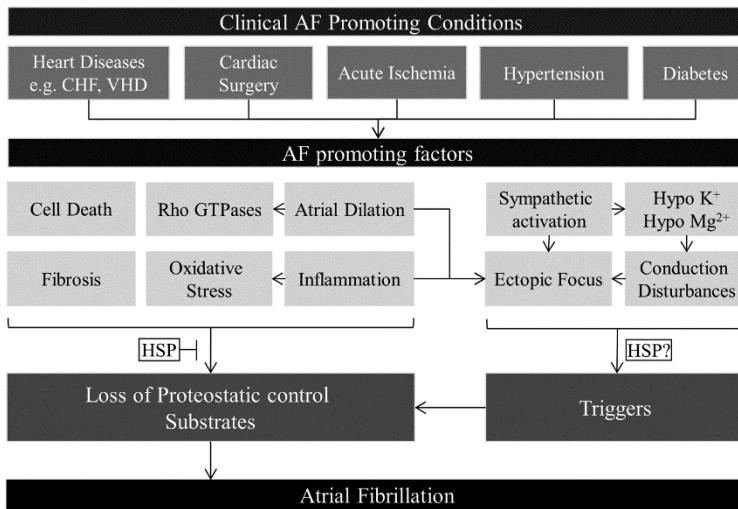


Figure 1: Overview of AF-promoting factors contributing to first-onset AF. Various clinical conditions induce AF promoting factors. These factors can induce triggers for AF or are responsible for the loss of proteostatic control, thereby inducing remodeling and creating a substrate for AF. Triggers will act on the vulnerable substrate to induce first-onset AF. Prevention and/or normalization of the cardiomyocyte proteostasis by inducing HSP expression could prevent AF substrate formation and prove an effective approach in preventing first-onset AF in response to various AF promoting factors.

Once AF is initiated, AF itself will induce further electrical and structural remodeling in a manner that contributes to AF maintenance and progression.²⁸ A conceptual model is depicted in Figure 2. Electrical remodeling resulting in shortening of action potential duration (APD), slowing of conduction and abnormal calcium handling will favor AF maintenance.²⁹ When AF persists, the calcium overload causes irreversible changes in structural remodeling, especially cardiomyocyte hibernation.³⁰⁻³² Hibernation is characterized by irreversible degradation of the myofibril structure (myolysis) and mitochondrial damage, implying impaired energy production and release of reactive oxygen species, which leads to contractile dysfunction.³³⁻³⁶ Other characteristics are redistribution of nuclear chromatin and pathological gene expression revealing a deficiency in healthy

cardiomyocyte proteostasis.^{30,34,37-40} While early electrical remodeling is reversible⁴¹, the derailment of proteostasis underlies irreversible structural remodeling and thereby AF progression.^{32,39,42-44} We and others identified various molecular mechanisms that underlie derailment of proteostasis and AF progression and recurrence.^{32,45-49}

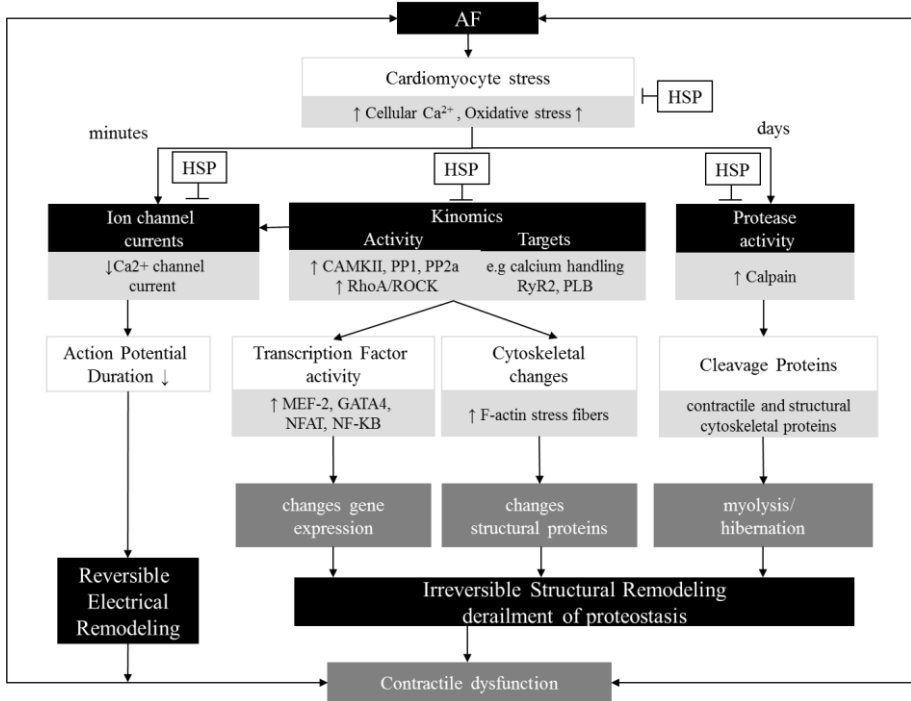


Figure 2: Overview of AF-induced derailment of cardiomyocyte proteostasis. AF induces time-related progressive myocyte remodeling. First, AF causes cellular Ca^{2+} overload and oxidative stress, which results in a direct inhibition of the L-type Ca^{2+} channel, shortening of action potential duration and contractile dysfunction. These changes have an early onset and are reversible. The early processes protect the cardiomyocyte against Ca^{2+} overload but at the expense of creating a substrate for persistent AF. When AF persists, derailment of proteostasis occurs via activation of calpain, kinases/phosphatases, RhoA-GTPase, and exhaustion of protective HSPs. The key modulators also activate each other. Derailment of proteostasis results in irreversible myolysis/hibernation, alterations in structural proteins and pathological gene expression, which are substrates for impaired contractile function and AF persistence. Upstream therapies are directed at modifying the substrate for AF progression. Normalization of the cardiomyocyte proteostasis by inducing HSP expression might represent an effective approach to manage clinical AF.

2.1. Ion channel currents and calcium handling

During AF, atrial cardiomyocytes are subjected to very rapid (400-600 times per min) and irregular firing, causing a rapid Ca^{2+} overload resulting in functional down-regulation of the L-type Ca^{2+} channel, which leads to shortening of the action potential duration and contractile dysfunction (hypocontractility), thus providing a further substrate for AF (Figure 2).^{45,50-53} Decreased I_{CaL} has been consistently found in atrial remodeling, which is believed to significantly contribute to electrical remodeling and AF progression.^{54,55} The

functional changes have a rapid onset following initiation of AF and are reversible.⁴¹ In addition to the L-type Ca^{2+} channel, also other channel currents are affected, as previously reviewed.²⁹ Ion channel currents are regulated by expression level, phosphorylation and redox status of the channel, all of which are altered during AF.^{29,56-59}

In dog atrial cardiomyocytes, tachypacing induced the activation of calcineurin via the Ca^{2+} /calmodulin system, which in turn changes cardiomyocyte proteostasis by stimulating nuclear translocation of NFAT, resulting in transcriptional and translational downregulation of the L-type calcium channel resulting in atrial remodeling and AF progression.⁵³ Phosphorylation status of ion channels are altered due to changes in activity of various kinases and phosphatases, such as enhanced CAMKII, PP1 and PP2a activity.^{29,60} Further, oxidative stress during AF can contribute to changes in redox status, thereby altering ion channel currents.^{29,58}

2.2. Kinome activity and target proteins

Kinome is a global description of kinases and kinase signaling. Various kinases and phosphatases become activated during AF and contribute to AF-induced remodeling and contractile dysfunction.^{48,60-64} In addition to modification of ion channel currents through (de)phosphorylation, also the function of other downstream target proteins are affected by the altered activity of kinases and phosphatases, including calcium handling proteins, such as RyR2 and PLB.⁶⁵⁻⁶⁸ Modification of calcium handling protein function will contribute to calcium overload and subsequent contractile dysfunction.⁶⁴ Furthermore, also kinases involved in post-translational modifications of structural proteins, such as actin, have been implicated in AF-induced remodeling. Tachypacing-induced activation of RhoA-GTPase and downstream Rho-kinase (ROCK), induces F-actin stress fiber formation (Figure 2).⁴⁸ Prevention of stress fiber formation by ROCK-inhibition or HSP expression prevented tachypacing-induced remodeling.⁴⁸ These findings imply a key role for alterations in kinome in causing derailment of proteostasis and progression of AF.

2.3. Protease activity

The cysteine protease calpain is activated during AF. Calpain is persistently activated by the AF-induced intracellular Ca^{2+} overload, which results in degradation of contractile and structural proteins^{32,69}, leading to myolysis, thereby further contributing to irreversible structural remodeling and AF progression.

Thus, AF-induced derailment of proteostasis includes changes in ion-channel function, kinome, and calpain activation and underlies reversible electrical remodeling and irreversible structural remodeling and thereby AF initiation and progression.

3. Heat shock proteins protect against AF initiation and progression

It has been recognized that heat shock transcription factor 1 (HSF1) is an important regulator of proteostasis by controlling the expression of major HSPs, including HSPB1 (HSP27), HSPA1A (HSP70) and HSPC1 (HSP90), that facilitate protein folding, localization and function.^{70,71} Induction of HSPs provides cytoprotective effects against stress induced derailment of proteostasis and is beneficial in various cardiac diseases (Table 1).^{46,48,70-83} Therefore, recent studies have investigated the cardioprotective potential of HSPs in AF, focusing on AF induction as well as progression.

Table 1; Major cardioprotective heat shock proteins, localization, expression and cardiac disease protective effects.

Family name	Protective member (alternative name)	Cardiac disease	localization	Cardiac Expression	References
HSPA	HSPA1A (HSP70)	ischemic heart disease, hypertrophy	cytosol	+++	72,73,78
DNAJ	DNAJA3 (HSC40)	dilated cardiomyopathy	cytosol/ nuclear	+++	76
	DNAJB5 (HSP40)	hypertrophy	cytosol/ nuclear	+++	82
HSPB	HSPB1 (HSP25, HSP27,HSP28)	AF, ischemic heart disease	cytosol	+++	46,48,74,79
	HSPB5 ($\alpha\beta$ Crystallin, CRYAB,CRYA1)	(dilated) cardiomyopathy	cytosol	++++	77,81
	HSPB6 (HSP20, p20)	AF, ischemic heart disease	cytosol	++	48,83
	HSPB7 (cvHSP)	AF	cytosol	+++++	48
	HSPB8 (HSP22,H11)	AF	cytosol	++	48
HSPD	HSPD1 (HSP60)	heart failure	mitochondria	++++	80
HSPC	HSPCA (HSP90)	ischemic heart disease	cytosol	++++	75

4. HSPs in the prevention of first-onset AF (primary prevention)

HSPs protect against a variety of AF-promoting factors contributing to first-onset AF (Figure 1). Protective effects of HSPs against cell death, fibrosis, Rho-GTPase activation, oxidative stress and inflammation have been described, indicating their potential in preventing loss of proteostatic control and formation of AF substrates.^{48,84-86} It is unclear if HSPs could affect the formation of triggers. However, since triggers need a vulnerable substrate to act on²², the prevention of AF substrate formation might be sufficient to protect against first-onset AF. Indeed in various models for first-onset AF, HSPs provide protection against AF-substrate formation and hence AF initiation. In a canine model (acute) atrial

ischemia related AF, GGA pretreatment induced HSPA1A expression and revealed protection against prolongation of effective refractory period (ERP) and atrial conduction abnormalities^{46,87}, thereby preventing AF initiation. These observations suggest that HSP induction may protect against some forms of AF in patients with coronary artery disease. Furthermore, a recent study in rats showed that induction of HSPA1A prevents both the Angiotensin II mediated atrial fibrosis and increased atrial vulnerability for AF induction.⁸⁵ The findings suggest HSPA1A to play a role in inhibiting the development of a non-cardiomyocyte substrate for AF induction. In two clinical studies, HSPA1A has been implicated as cardioprotective, showing a correlation between HSPA1A atrial expression levels and reduced incidence of post-operative AF in patients in sinus rhythm undergoing cardiac surgery.^{88,89}

In addition to HSPA1A, other HSPs might be involved in primary prevention of AF. In patients with AF, increased mitochondrial HSP expression levels, i.e. HSPD1, HSPE1 and mortalin (HSPA9B)⁹⁰ have been reported. In addition, increased HSPD1 antibody levels in the serum of patients have been associated with the occurrence of post-operative AF⁹¹, suggesting HSPD1 as a marker for mitochondrial and cardiac damage and subsequent increased risk for AF. Increased expression of mitochondrial chaperones could contribute to an increased protection against oxidative stress. Therefore, these HSP family members might contribute to survival of cardiomyocytes by maintaining mitochondrial integrity and capacity for ATP generation. To date, however, several studies showed opposing correlations between the expression of mitochondrial HSPs and AF⁹⁰⁻⁹², thereby obscuring their involvement in protection against AF.

5. HSPs in the prevention of AF progression and recurrence (secondary prevention)

Various *in vitro* and *in vivo* models for tachypacing-induced AF progression identified HSPs to protect against the derailment of proteostasis and cardiomyocyte remodeling. In tachypaced HL-1 atrial cardiomyocytes for AF, a general HSP induction via a mild heat shock or by a HSP-inducing drug geranylgeranylacetone (GGA), conserved cardiomyocyte proteostasis during tachypacing and protected against subsequent electrical, contractile and structural remodeling.^{15,46} Furthermore, in canine models for AF progression, GGA pretreatment induced HSP (HSPA1A and HSPB1) expression and revealed protective effects against shortening of ERP, shortening of APD, reductions in L-type Ca²⁺ current and AF progression.^{46,87} Also, in clinical studies, a potent HSR and high HSPB1 levels have been associated with restoration of normal sinus rhythm in patients with permanent AF after mitral valve surgery.¹⁸ Two other studies comparing paroxysmal versus persistent AF and sinus rhythm, found an inverse correlation between HSPB1 atrial expression and AF duration and extend of myolysis.^{15,92} Suggesting, a temporary activation of the HSR during

a short duration of AF but exhaustion in time related to the duration of AF. Consequently, cardiomyocytes lose the ability for proteostatic control, inducing remodeling, which will result in AF progression and recurrence.

Further studies investigated the role of individual HSPs in protection against tachypacing-induced remodeling. HSPB1, and not HSPA1A, was found to play an important role, as its exclusive overexpression appears sufficient to protect against tachypacing-induced remodeling, comparable to GGA pre-treatment.^{15,46} Conversely, the protective effect of a general HSR or GGA pre-treatment on tachypacing-induced changes was annihilated by a selective knockdown of HSPB1. However, in addition to HSPB1, also other HSPB family members (HSPB6, HSPB7 and HSPB8) protect against AF-induced structural remodeling independently from HSPB1.⁴⁸ Hence, multiple HSPB family members prevent against AF-induced cardiomyocyte remodeling and AF progression by preserving cell proteostasis, thereby demonstrating their therapeutic potential in AF.

Taken together, there seems to be a strong case for induction of HSPs to prevent AF initiation, recurrences and progression, by attenuation of electrical, contractile and structural cardiomyocyte remodeling. There are strong indications that this effect is via normalization of cell proteostasis.

6. Mode of action for HSPs to normalize proteostasis

It has been recognized that HSPs protect against derailment of proteostasis by preventing cardiomyocyte remodeling at different stages (Figure 1 and 2). The exact mechanisms in prevention of AF initiation (primary prevention) and recurrences (secondary prevention) are not known, but are likely due to HSP regulated protection against various AF promoting factors that induce the substrate for AF initiation and progression.

6.1. Ion channel currents

An ion channel current is dependent on the expression level, phosphorylation and redox status of the channel^{29,58,93}, as well as the stability of the cytoskeleton⁹⁴ and Rho-GTPase activity.⁹⁵ The HSP-inducing drug GGA previously showed protective effects against tachypacing-induced reductions in L-type Ca²⁺ current and shortening of APD.⁴⁶ Furthermore, several studies have shown protective effect of HSPs on almost all of these regulating factors. HSPs are known to interact and, in some cases, inhibit kinases and phosphatases, whose activity is altered during AF^{83,96-100}, thereby potentially preventing or normalizing the phosphorylation status of ion channels, especially L-type Ca²⁺ channel.⁶¹ Furthermore, several HSPs (including HSPB1) were shown to provide protection against oxidative stress, thereby potentially preventing or restoring the redox status of the ion channels.¹⁰¹ If HSPs can influence the expression levels of ion channels is currently not

known. Lastly, also the stability of the actin cytoskeleton and Rho-GTPase activity are regulated by the small HSP family members (see below).^{46,48,49,102-105} The findings reveal a protective role of HSP against AF-induced changes in ion channel current, including reductions in the L-type Ca^{2+} current.

6.2. Kinome activity and target proteins

Various kinases and phosphatases reveal changed activity during AF, which contributes to cardiomyocyte remodeling depending on their target proteins.^{48,60-64} In addition to ion-channels, known targets are transcription factors, various calcium handling proteins and the actin cytoskeleton. Changes in transcription factor phosphorylation, regulates gene expression and hence can induce an altered gene expression profile, possibly contributing to cardiomyocyte hibernation. Interestingly, HSPB1 was shown to interact with certain (downstream) kinases, such as I κ B kinase and c-Jun N-terminal kinase (JNK), thereby suppressing activation of the transcription factor NF- κ B.^{106,107} Interestingly, these kinases have also been found to be modulated during AF.^{108,109} In addition, HSPB1 is known to interact with other kinases and phosphatases and thereby might prevent the activation of other downstream transcription factors.^{83,96-100}

Changes in phosphorylation status of calcium handling proteins will affect the calcium homeostasis in cardiomyocytes. It is generally accepted that AF-induced abnormalities in intracellular Ca^{2+} handling leads to atrial cardiomyocyte stress and induces remodeling that contributes to the progression of AF.^{53,110} A calcium overload can be caused by an increase in L-type Ca^{2+} channel activity, or a changed activity of calcium handling proteins such as RyR2, SR Ca^{2+} ATPases or $\text{Na}^+/\text{Ca}^{2+}$ exchanger. These rapid changes in activity of proteins involved in calcium handling are modulated by kinases and/or phosphatases, including CAMKII and PP1, of which the activities are increased during AF.^{61,111} Interestingly, studies showed that HSPs interact with CaMKII⁹⁹, calcineurin⁹⁷ and PP1.^{98,83} Furthermore, HSPB6 was shown to inhibit PP1 activity.¹⁰⁰ Also, HSPs increase SR Ca^{2+} ATPase activity and stimulate both the reuptake of Ca^{2+} into the SR and the extrusion of Ca^{2+} out of the cardiomyocyte via $\text{Na}^+/\text{Ca}^{2+}$ exchanger.^{112,113} These findings suggest that HSPs can protect against (tachypacing-induced) changes in calcium handling proteins, resulting in attenuation of AF progression.

AF is known to activate RhoA-GTPase and ROCK and induces subsequent F-actin stress fiber formation contributing to contractile dysfunction.⁴⁸ Several HSPB family members (HSPB1, HSPB6 and HSPB7) were recently shown to bind to actin and prevent F-actin stress fiber formation downstream of RhoA/ROCK. HSPB1 and HSPB6 even promoted actin stress fiber disassembly. HSPB8 did not directly bind actin, but instead inhibited upstream RhoA GTPase activation, thereby preventing F-actin stress fiber

formation.⁴⁸ As most HSPB family members are known to protect the actin cytoskeleton from remodeling, this action likely represents an important mechanism by which HSPBs attenuate AF-induced derailment of proteostasis and cardiomyocyte remodeling.

6.3. HSPs and oxidative stress

Interestingly, in AF patients, an increase in oxidative stress markers has been observed and anti-inflammatory or anti-oxidant treatment with glucocorticoids and statins¹¹⁴⁻¹¹⁷ suppressed atrial remodeling and have shown some clinical value in prevention of post-surgery AF (primary prevention)⁵, substantiating a role for oxidative stress in AF-induced remodeling. Glucocorticoids and statins have been reported to induce several HSPs (HSPB1, HSPB5 and HSPA1A)^{118,119}, leaving open the possibility that part of their protective pleiotropic effects is due to overexpression of HSPs. HSP induction can provide protection against oxidative stress by several mechanisms. HSPB1 is known to regulate the redox status of cardiomyocytes by maintaining glutathione in its reduced form, thus decreasing the amount of reactive oxygen species (ROS) produced in cells exposed to oxidative stress or tumor necrosis factor TNFalpha.¹²⁰ HSPB1 may therefore prevent tachypacing-induced alterations in redox status of cardiomyocytes and thereby preserve cell proteostasis and electrophysiological and contractile function of the cardiomyocyte in AF. In addition to alterations in redox state, oxidative stress can also contribute to actin cytoskeleton instability, resulting in impairment of cardiomyocyte contractile function. Several members of the HSPB-family were found to bind the actin filaments and prevent their disruption in response to various stresses, including AF.^{46,49,102-105}

7. Therapeutic potential of HSP inducing drugs in AF

Pharmacological approaches preventing the substrate for AF are being studied, with the hope that they might be useful therapeutic agents in treating AF.¹²¹ So far, the efficacy of commonly used drugs, including glucocorticoids and statins, on remodeling is limited¹²¹ and (serious) adverse effects have been reported, indicating the need for more effective therapeutic agents with less adverse effects. Since derailment of cellular proteostasis results in cardiomyocyte remodeling and AF progression and derailment is counteracted by HSPs, pharmacological induction of the HSR seems to represent a key target for upstream therapy.

Currently, GGA represents the most promising compound for the pharmacological induction of HSPs. Until now it is the most efficacious HSP boosting drug.¹²² Furthermore, in contrast to other HSP inducers, GGA is a nontoxic compound shown to be capable of inducing HSP expression in various tissues, including gastric mucosa, intestine, liver, myocardium, retina, kidney, and central nervous system. In addition GGA is used clinically in Japan since 1984 as an antiulcer drug¹²³ and no serious adverse reactions have been

reported.¹²⁴⁻¹²⁷ GGA rapidly induces HSP expression (HSPB, HSPA1A, HSPC family members) via activation of the heat shock transcription factor HSF1, in response to a variety of stresses, whereas its effect is weaker under non-stress conditions, providing its main effect when and where needed.^{128,129}

The protective effect of GGA-induced HSP expression on early and late remodeling, suggests that it has value in the prevention of clinical AF, although this still needs to be assessed in clinical trials.^{46,87,130} So far, the protective action of GGA has been established regarding electrical, contractile and structural remodeling in *in vitro* HL-1 and dog atrial cardiomyocytes and in *in vivo* dog models for AF. GGA also has beneficial effects in AF of different origin, as observed in AF induced by congestive heart failure and acute ischemia.^{87,116} The broad protective effects of GGA against AF-related derailment of proteostasis and atrial remodeling suggest that inducers of the HSR have substantial therapeutic value for clinical AF. Other drugs that induce HSP expression, such as bimosamol, atorvastatin, cyclosporine A, dexamethasone, still need to be tested for their protective effects against AF-induced remodeling. Nevertheless, their therapeutic potential in other cardiac diseases, such as ischemic heart disease, have already been documented.^{74,131-135}

In summary, AF results in a derailment of cardiomyocyte proteostasis by inducing reversible electrical and irreversible structural remodeling. There is strong evidence that induction of HSPs, in particular HSPB family members, normalizes proteostasis and thereby prevents electrical and structural remodeling. Known upstream targets for HSP protection include L-type Ca²⁺ channel, calcium handling proteins, calpain, RhoA-GTPase and F-actin stress fibers. Ultimately, the induction of HSPs, by proteostasis regulators such as GGA, may prevent the occurrence of AF (primary prevention) and may contribute to enhance therapeutic efficacy and treatment options for patient with AF in delaying progression towards persistent AF and/or improve the outcome of cardioversion (secondary prevention).

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REFERENCES

1. Wang TJ, Parise H, Levy D, D'Agostino RB, Wolf PA, Vasan RS, Benjamin EJ. Obesity and the risk of new-onset atrial fibrillation. *JAMA*. 2004; 292: 2471-2477.
2. Watanabe H, Tanabe N, Watanabe T, Darbar D, Roden DM, Sasaki S, Aizawa Y. Metabolic syndrome and risk of development of atrial fibrillation: the Niigata preventive medicine study. *Circulation*. 2008; 117: 1255-1260.
3. Feinberg WM, Blackshear JL, Laupacis A, Kronmal R, Hart RG. Prevalence, age distribution, and gender of patients with atrial fibrillation: analysis and implications. *Arch Intern Med*. 1995; 155: 469-473.
4. Van Gelder I, Crijns H, Tieleman R, De Kam P, Gosseling A, Verheugt F, Lie K. Value and limitation of electrical cardioversion in patients with chronic atrial fibrillation—importance of arrhythmia risk factors and oral anticoagulation. *Arch Intern Med*. 1996; 156: 2585-2592.
5. Camm AJ, Kirchhof P, Lip GYH, Schotten U, Savelieva I, Ernst S, Van Gelder IC, Al-Attar N, Hindricks G, Prendergast B, Heidbuchel H, Alfieri O, Angelini A, Atar D, Colonna P, De Caterina R, De Sutter J, Goette A, Gorenek B, Heldal M, Hohloser SH, Kolh P, Le Heuzey J, Ponikowski P, Rutten FH, European Assoc Cardio-Thoracic. Guidelines for the management of atrial fibrillation The Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). *Eur Heart J*. 2010; 31: 2369-2429.
6. Savelieva I, Kakouros N, Kourliouros A, Camm AJ. Upstream therapies for management of atrial fibrillation: review of clinical evidence and implications for European Society of Cardiology guidelines. Part II: secondary prevention. *Europace*. 2011; 13: 610-625.
7. Van Gelder I, Smit M, Alings M, Crijns H. Upstream therapy in patients with early atrial fibrillation. *Netherlands Heart Journal*. 2010; 18: 522-523.
8. Savelieva I, Kakouros N, Kourliouros A, Camm AJ. Upstream therapies for management of atrial fibrillation: review of clinical evidence and implications for European Society of Cardiology guidelines. Part I: primary prevention. *Europace*. 2011; 13: 308-328.
9. Nattel S, Burstein B, Dobrev D. Atrial Remodeling and Atrial Fibrillation Mechanisms and Implications. *Circulation-Arrhythmia and Electrophysiology*. 2008; 1: 62-73.
10. Brundel BJ, Van Gelder IC, Henning RH, Tieleman RG, Tuinenburg AE, Wietes M, Grandjean JG, Van Gilst WH, Crijns HJ. Ion channel remodeling is related to intraoperative atrial effective refractory periods in patients with paroxysmal and persistent atrial fibrillation. *Circulation*. 2001; 103: 684-690.
11. Brundel BJM, Ausma J, van Gelder IC, Van Der Want JLL, van Gilst WH, Crijns HJGM, Henning RH. Activation of proteolysis by calpains and structural changes in human paroxysmal and persistent atrial fibrillation. *Cardiovasc Res*. 2002; 54: 380-389.
12. Brundel BJM, Shiroshita-Takeshita A, Qi X, Yeh Y, Chartier D, van Gelder IC, Henning RH, Kampinga HH, Nattel S. Induction of heat shock response protects the heart against atrial fibrillation. *Circ Res*. 2006; 99: 1394-1402.
13. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science*. 2008; 319: 916-919.
14. Westerheide SD, Morimoto RI. Heat shock response modulators as therapeutic tools for diseases of protein conformation. *J Biol Chem*. 2005; 280: 33097-33100.
15. Brundel BJ, Henning RH, Ke L, van Gelder IC, Crijns HJ, Kampinga HH. Heat shock protein upregulation protects against pacing-induced myolysis in HL-1 atrial myocytes and in human atrial fibrillation. *J Mol Cell Cardiol*. 2006; 41: 555-562.
16. Brundel BJ, Ke L, Dijkhuis AJ, Qi X, Shiroshita-Takeshita A, Nattel S, Henning RH, Kampinga HH. Heat shock proteins as molecular targets for intervention in atrial fibrillation. *Cardiovasc Res*. 2008; 78: 422-428.
17. Sakabe M, Shiroshita-Takeshita A, Maguy A, Brundel BJ, Fujiki A, Inoue H, Nattel S. Effects of a heat shock protein inducer on the atrial fibrillation substrate caused by acute atrial ischaemia. *Cardiovasc Res*. 2008; 78: 63-70.
18. Cao H, Xue L, Xu X, Wu Y, Zhu J, Chen L, Chen D, Chen Y. Heat shock proteins in stabilization of spontaneously restored sinus rhythm in permanent atrial fibrillation patients after mitral valve surgery. *Cell Stress and Chaperones*. 2011; 16: 517-528.

19. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. *Circulation*. 1998; 98: 946-952.
20. Kannel WB, Wolf PA, Benjamin EJ, Levy D. Prevalence, incidence, prognosis, and predisposing conditions for atrial fibrillation: population-based estimates. *Am J Cardiol*. 1998; 82: 2N-9N.
21. Chelazzi C, Villa G, De Gaudio AR. Postoperative atrial fibrillation. *ISRN Cardiol*. 2011; 2011: 203179.
22. Allesie MA, de Groot NM, Houben RP, Schotten U, Boersma E, Smeets JL, Crijns HJ. Electropathological substrate of long-standing persistent atrial fibrillation in patients with structural heart disease: longitudinal dissociation. *Circ Arrhythm Electrophysiol*. 2010; 3: 606-615.
23. de Groot NM, Houben RP, Smeets JL, Boersma E, Schotten U, Schalij MJ, Crijns H, Allesie MA. Electropathological substrate of longstanding persistent atrial fibrillation in patients with structural heart disease: epicardial breakthrough. *Circulation*. 2010; 122: 1674-1682.
24. Sah VP, Minamisawa S, Tam SP, Wu TH, Dorn GW, 2nd, Ross J, Jr, Chien KR, Brown JH. Cardiac-specific overexpression of RhoA results in sinus and atrioventricular nodal dysfunction and contractile failure. *J Clin Invest*. 1999; 103: 1627-1634.
25. Dudley SC, Jr, Hoch NE, McCann LA, Honeycutt C, Diamandopoulos L, Fukui T, Harrison DG, Dikalov SI, Langberg J. Atrial fibrillation increases production of superoxide by the left atrium and left atrial appendage: role of the NADPH and xanthine oxidases. *Circulation*. 2005; 112: 1266-1273.
26. Burstein B, Nattel S. Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. *J Am Coll Cardiol*. 2008; 51: 802-809.
27. Nattel S, Burstein B, Dobrev D. Atrial remodeling and atrial fibrillation: mechanisms and implications. *Circ Arrhythm Electrophysiol*. 2008; 1: 62-73.
28. Wijffels MC, Kirchhof CJ, Dorland R, Allesie MA. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. *Circulation*. 1995; 92: 1954-1968.
29. Dobrev D, Voigt N. Ion channel remodelling in atrial fibrillation. 2011; .
30. Ausma J, Wijffels M, van Eys G, Koide M, Ramaekers F, Allesie M, Borgers M. Dedifferentiation of atrial cardiomyocytes as a result of chronic atrial fibrillation. *Am J Pathol*. 1997; 151: 985-997.
31. Ausma J, Duimel H, Borgers M, Allesie MA. Recovery of structural remodeling after cardioversion of chronic atrial fibrillation. *Circulation*. 2000; 102: 153-153.
32. Brundel BJM, Ausma J, van Gelder IC, Van Der Want JLL, van Gilst WH, Crijns HJGM, Henning RH. Activation of proteolysis by calpains and structural changes in human paroxysmal and persistent atrial fibrillation. *Cardiovasc Res*. 2002; 54: 380-389.
33. Vanoverschelde JL, Wijns W, Depre C, Essamri B, Heyndrickx GR, Borgers M, Bol A, Melin JA. Mechanisms of chronic regional posts ischemic dysfunction in humans. New insights from the study of noninfarcted collateral-dependent myocardium. *Circulation*. 1993; 87: 1513-1523.
34. Ausma J, Wijffels M, Thone F, Wouters L, Allesie M, Borgers M. Structural changes of atrial myocardium due to sustained atrial fibrillation in the goat. *Circulation*. 1997; 96: 3157-3163.
35. Sherman AJ, Klocke FJ, Decker RS, Decker ML, Kozlowski KA, Harris KR, Hedjbeli S, Yaroshenko Y, Nakamura S, Parker MA, Checchia PA, Evans DB. Myofibrillar disruption in hypocontractile myocardium showing perfusion-contraction matches and mismatches. *Am J Physiol Heart Circ Physiol*. 2000; 278: H1320-34.
36. Bito V, Heinzel FR, Weidemann F, Dommke C, van der Velden J, Verbeke E, Claus P, Bijnens B, De Scheerder I, Stienen GJ, Sutherland GR, Sipido KR. Cellular mechanisms of contractile dysfunction in hibernating myocardium. *Circ Res*. 2004; 94: 794-801.
37. Ausma J, Fürst D, Thon éF, Shivalkar B, Flameng W, Weber K, Ramaekers F, Borgers M. Molecular changes of titin in left ventricular dysfunction as a result of chronic hibernation. *J Mol Cell Cardiol*. 1995; 27: 1203-1212.
38. Ausma J, Thone F, Dispensy G, Flameng W, Vanoverschelde J, Ramaekers F, Borgers M. Dedifferentiated cardiomyocytes from chronic hibernating myocardium are ischemia-tolerant. *Mol Cell Biochem*. 1998; 186: 159-168.

39. Allesie M, Ausma J, Schotten U. Electrical, contractile and structural remodeling during atrial fibrillation. *Cardiovasc Res.* 2002; 54: 230-246.
40. Thijssen VL, Ausma J, Gorza L, van der Velden HM, Allesie MA, Van Gelder IC, Borgers M, van Eys GJ. Troponin I isoform expression in human and experimental atrial fibrillation. *Circulation.* 2004; 110: 770-775.
41. Schotten U, Duytschaever M, Ausma J, Eijssbouts S, Neuberger HR, Allesie M. Electrical and contractile remodeling during the first days of atrial fibrillation go hand in hand. *Circulation.* 2003; 107: 1433-1439.
42. Ausma J, van der Velden HMW, Lenders MH, van Ankeren EP, Jongsma HJ, Ramaekers F, Borgers M, Allesie MA. Reverse structural and gap-junctional remodeling after prolonged atrial fibrillation in the goat. *Circulation.* 2003; 107: 2051-2058.
43. Cha TJ, Ehrlich JR, Zhang L, Shi YF, Tardif JC, Leung TK, Nattel S. Dissociation between ionic remodeling and ability to sustain atrial fibrillation during recovery from experimental congestive heart failure. *Circulation.* 2004; 109: 412-418.
44. Todd DM, Fynn SP, Walden AP, Hobbs WJ, Arya S, Garratt CJ. Repetitive 4-week periods of atrial electrical remodeling promote stability of atrial fibrillation: time course of a second factor involved in the self-perpetuation of atrial fibrillation. *Circulation.* 2004; 109: 1434-1439.
45. Brundel BJ, Van Gelder IC, Henning RH, Tieleman RG, Tuinenburg AE, Wietses M, Grandjean JG, Van Gilst WH, Crijns HJ. Ion channel remodeling is related to intraoperative atrial effective refractory periods in patients with paroxysmal and persistent atrial fibrillation. *Circulation.* 2001; 103: 684-90.
46. Brundel BJ, Shiroshita-Takeshita A, Qi X, Yeh YH, Chartier D, van Gelder IC, Henning RH, Kampinga HH, Nattel S. Induction of heat shock response protects the heart against atrial fibrillation. *Circ Res.* 2006; 99: 1394-402.
47. Ke L, Qi XY, Dijkhuis AJ, Chartier D, Nattel S, Henning RH, Kampinga HH, Brundel BJ. Calpain mediates cardiac troponin degradation and contractile dysfunction in atrial fibrillation. *Journal of molecular and cellular cardiology.* 2008; 45: 685-93.
48. Ke L, Meijering RA, Hoogstra-Berends F, Mackovicova K, Vos MJ, Van Gelder IC, Henning RH, Kampinga HH, Brundel BJ. HSPB1, HSPB6, HSPB7 and HSPB8 protect against RhoA GTPase-induced remodeling in tachypaced atrial myocytes. *PLoS One.* 2011; 6: e20395.
49. Zhang D, Ke L, Mackovicova K, Der Want JJLV, Sibon O, Tanguay RM, Morrow G, Henning RH, Kampinga HH, Brundel BJJM. Effects of different small HSPB members on contractile dysfunction and structural changes in a *Drosophila melanogaster* model for Atrial Fibrillation. *J Mol Cell Cardiol.* 2011; .
50. Goette A, Honeycutt C, Langberg JJ. Electrical remodeling in atrial fibrillation. Time course and mechanisms. *Circulation.* 1996; 94: 2968-2974.
51. Leistad E, Borgers M, Christensen G. Atrial contractile dysfunction after short-term atrial fibrillation can be explained by changes in intracellular calcium, but not by atrial ischemia. *Circulation.* 1996; 94: 2254-2254.
52. Ausma J, Dispersyn GD, Duimel H, Thone F, Ver Donck L, Allesie MA, Borgers M. Changes in ultrastructural calcium distribution in goat atria during atrial fibrillation. *J Mol Cell Cardiol.* 2000; 32: 355-364.
53. Qi XY, Yeh YH, Xiao L, Burstein B, Maguy A, Chartier D, Villeneuve LR, Brundel BJ, Dobrev D, Nattel S. Cellular signaling underlying atrial tachycardia remodeling of L-type calcium current. *Circ Res.* 2008; 103: 845-54.
54. van Wagoner D, Lamorgese M, Kirian P, Cheng Y, Efimov I, Mazgalev T, Nerbonne J. Calcium current density is reduced in atrial myocytes isolated from patients in chronic atrial fibrillation. 1997; 96: 995-995.
55. Yue L, Feng J, Gaspo R, Li GR, Wang Z, Nattel S. Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. *Circ Res.* 1997; 81: 512-525.
56. Brundel BJ, van Gelder IC, Henning RH, Tuinenburg AE, Deelman LE, Tieleman RG, Grandjean JG, van Gilst WH, Crijns HJ. Gene expression of proteins influencing the calcium homeostasis in patients with persistent and paroxysmal atrial fibrillation. *Cardiovasc Res.* 1999; 42: 443-54.
57. Van Gelder IC, Brundel BJ, Henning RH, Tuinenburg AE, Tieleman RG, Deelman L, Grandjean JG, De Kam PJ, Van Gilst WH, Crijns HJ. Alterations in gene expression of proteins involved in the calcium handling in patients with atrial fibrillation. *J Cardiovasc Electrophysiol.* 1999; 10: 552-560.

58. Wang Z. Role of redox state in modulation of ion channel function by fatty acids and phospholipids. *Br J Pharmacol.* 2003; 139: 681-683.
59. Voigt N, Dobrev D. Ion channel remodelling in atrial fibrillation. 2011; .
60. Anderson ME. Calmodulin kinase and L-type calcium channels: A recipe for arrhythmias? *Trends Cardiovasc Med.* 2004; 14: 152-161.
61. Christ T, Boknik P, Wohrl S, Wettwer E, Graf EM, Bosch RF, Knaut M, Schmitz W, Ravens U, Dobrev D. L-type Ca²⁺ current downregulation in chronic human atrial fibrillation is associated with increased activity of protein phosphatases. *Circulation.* 2004; 110: 2651-2657.
62. El-Armouche A, Boknik P, Eschenhagen T, Carrier L, Knaut M, Ravens U, Dobrev D. Molecular determinants of altered Ca²⁺ handling in human chronic atrial fibrillation. *Circulation.* 2006; 114: 670-680.
63. Greiser M, Halaszovich CR, Frechen D, Boknik P, Ravens U, Dobrev D, Lückhoff A, Schotten U. Pharmacological evidence for altered src kinase regulation of I_{Ca,L} in patients with chronic atrial fibrillation. *Naunyn Schmiedebergs Arch Pharmacol.* 2007; 375: 383-392.
64. Dobrev D, Nattel S. Calcium handling abnormalities in atrial fibrillation as a target for innovative therapeutics. *J Cardiovasc Pharmacol.* 2008; 52: 293-299.
65. Schwinger RH, Münch G, Bäck B, Karczewski P, Krause E, Erdmann E. Reduced Ca²⁺-sensitivity of SERCA 2a in failing human myocardium due to reduced serin-16 phospholamban phosphorylation. *J Mol Cell Cardiol.* 1999; 31: 479-491.
66. Hagemann D, Xiao R. Dual site phospholamban phosphorylation and its physiological relevance in the heart. *Trends Cardiovasc Med.* 2002; 12: 51-56.
67. Bers D. Cardiac ryanodine receptor phosphorylation: target sites and functional consequences. *Biochem J.* 2006; 396: e1-e3.
68. Carter S, Pitt S, Colyer J, Sitsapesan R. Ca²⁺-Dependent Phosphorylation of RyR2 Can Uncouple Channel Gating from Direct Cytosolic Ca²⁺ Regulation. *J Membr Biol.* 2011; 240: 21-33.
69. Ke L, Qi XY, Dijkhuis AJ, Chartier D, Nattel S, Henning RH, Kampinga HH, Brundel BJJM. Calcain mediates cardiac troponin degradation and contractile dysfunction in atrial fibrillation. *J Mol Cell Cardiol.* 2008; 45: 685-693.
70. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science.* 2008; 319: 916-919.
71. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem.* 2009; 78: 959-991.
72. Marber MS, Mestrlil R, Chi SH, Sayen MR, Yellon DM, Dillmann WH. Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. *J Clin Invest.* 1995; 95: 1446-1456.
73. Plumier JC, Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G, Pagoulatos GN. Transgenic mice expressing the human heat shock protein 70 have improved post-ischemic myocardial recovery. *J Clin Invest.* 1995; 95: 1854-1860.
74. Efthymiou CA, Mocanu MM, de Bellerocche J, Wells DJ, Latchmann DS, Yellon DM. Heat shock protein 27 protects the heart against myocardial infarction. *Basic Res Cardiol.* 2004; 99: 392-394.
75. Kupatt C, Dessy C, Hinkel R, Raake P, Daneau G, Bouzin C, Boekstegers P, Feron O. Heat shock protein 90 transfection reduces ischemia-reperfusion-induced myocardial dysfunction via reciprocal endothelial NO synthase serine 1177 phosphorylation and threonine 495 dephosphorylation. *Arterioscler Thromb Vasc Biol.* 2004; 24: 1435-1441.
76. Hayashi M, Imanaka-Yoshida K, Yoshida T, Wood M, Fearn C, Tataka RJ, Lee J. A crucial role of mitochondrial Hsp40 in preventing dilated cardiomyopathy. *Nat Med.* 2006; 12: 128-132.
77. Inagaki N, Hayashi T, Arimura T, Koga Y, Takahashi M, Shibata H, Teraoka K, Chikamori T, Yamashina A, Kimura A. α B-crystallin mutation in dilated cardiomyopathy. *Biochem Biophys Res Commun.* 2006; 342: 379-386.

78. Kim YK, Suarez J, Hu Y, McDonough PM, Boer C, Dix DJ, Dillmann WH. Deletion of the inducible 70-kDa heat shock protein genes in mice impairs cardiac contractile function and calcium handling associated with hypertrophy. *Circulation*. 2006; 113: 2589-2597.
79. Kwon JH, Kim J, Lee K, Kang S, Chung N, Jang Y, Chung JH. Protective effect of heat shock protein 27 using protein transduction domain-mediated delivery on ischemia/reperfusion heart injury. *Biochem Biophys Res Commun*. 2007; 363: 399-404.
80. Lin L, Kim SC, Wang Y, Gupta S, Davis B, Simon SI, Torre-Amione G, Knowlton AA. HSP60 in heart failure: abnormal distribution and role in cardiac myocyte apoptosis. *Am J Physiol Heart Circ Physiol*. 2007; 293: H2238-47.
81. Rajasekaran NS, Connell P, Christians ES, Yan L, Taylor RP, Orosz A, Zhang XQ, Stevenson TJ, Peshock RM, Leopold JA. Human α B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. *Cell*. 2007; 130: 427-439.
82. Ago T, Liu T, Zhai P, Chen W, Li H, Molkentin JD, Vatner SF, Sadoshima J. A redox-dependent pathway for regulating class II HDACs and cardiac hypertrophy. *Cell*. 2008; 133: 978-993.
83. Fan G, Kranias EG. Small heat shock protein 20 (HspB6) in cardiac hypertrophy and failure. *J Mol Cell Cardiol*. 2011; 51: 574-577.
84. Arya R, Mallik M, Lakhota SC. Heat shock genes-integrating cell survival and death. *J Biosci*. 2007; 32: 595-610.
85. Wakisaka O, Takahashi N, Shinohara T, Ooie T, Nakagawa M, Yonemochi H, Hara M, Shimada T, Saikawa T, Yoshimatsu H. Hyperthermia treatment prevents angiotensin II-mediated atrial fibrosis and fibrillation via induction of heat-shock protein 72. *J Mol Cell Cardiol*. 2007; 43: 616-626.
86. Jones Q, S Voegeli T, Li G, Chen Y, William Currie R. Heat shock proteins protect against ischemia and inflammation through multiple mechanisms. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy)*. 2011; 10: 247-259.
87. Sakabe M, Shiroshita-Takeshita A, Maguy A, Brundel BJ, Fujiki A, Inoue H, Nattel S. Effects of a heat shock protein inducer on the atrial fibrillation substrate caused by acute atrial ischaemia. *Cardiovasc Res*. 2008; 78: 63-70.
88. Rammos KS, Koullias GJ, Hassan MO, Argyrakis NP, Voucharas CG, Scarupa SJ, Cowte TG. Low preoperative HSP70 atrial myocardial levels correlate significantly with high incidence of postoperative atrial fibrillation after cardiac surgery. *Vascular*. 2002; 10: 228-232.
89. Mandal K, Torsney E, Poloniecki J, Camm AJ, Xu Q, Jahangiri M. Association of high intracellular, but not serum, heat shock protein 70 with postoperative atrial fibrillation. *Ann Thorac Surg*. 2005; 79: 865-871.
90. Kirmanoglou K, Hannekem A, Schäfer AE. Expression of mortalin in patients with chronic atrial fibrillation. *Basic Res Cardiol*. 2004; 99: 404-408.
91. Oc M, Ucar HI, Pinar A, Akbulut B, Oc B, Akinci SB, Akyon Y, Kanbak M, Boke E, Dogan R. Heat shock protein 60 antibody. A new marker for subsequent atrial fibrillation development. *Saudi Med J*. 2007; 28: 844-847.
92. Yang M, Tan H, Cheng L, He M, Wei Q, Tanguay RM, Wu T. Expression of heat shock proteins in myocardium of patients with atrial fibrillation. *Cell Stress Chaperones*. 2007; 12: 142-150.
93. Hara Y, Wakamori M, Ishii M, Maeno E, Nishida M, Yoshida T, Yamada H, Shimizu S, Mori E, Kudoh J. LTRPC2 Ca²⁺-permeable channel activated by changes in redox status confers susceptibility to cell death. *Mol Cell*. 2002; 9: 163-173.
94. Sadoshima J, Takahashi T, Jahn L, Izumo S. Roles of mechano-sensitive ion channels, cytoskeleton, and contractile activity in stretch-induced immediate-early gene expression and hypertrophy of cardiac myocytes. *Proc Natl Acad Sci U S A*. 1992; 89: 9905-9909.
95. Pochynyuk O, Stockand JD, Staruschenko A. Ion channel regulation by Ras, Rho, and Rab small GTPases. *Exp Biol Med (Maywood)*. 2007; 232: 1258-1265.
96. Ding XZ, Tsokos GC, Kiang JG. Overexpression of HSP-70 inhibits the phosphorylation of HSF1 by activating protein phosphatase and inhibiting protein kinase C activity. *FASEB J*. 1998; 12: 451-459.

97. Lakshmikuttyamma A, Selvakumar P, Anderson DH, Datla RS, Sharma RK. Molecular cloning of bovine cardiac muscle heat-shock protein 70 kDa and its phosphorylation by cAMP-dependent protein kinase in vitro. *Biochemistry (N Y)*. 2004; 43: 13340-13347.
98. Dobrev D. Electrical remodeling in atrial fibrillation. *Herz*. 2006; 31: 108-112.
99. Peng W, Zhang Y, Zheng M, Cheng H, Zhu W, Cao CM, Xiao RP. Cardioprotection by CaMKII-deltaB is mediated by phosphorylation of heat shock factor 1 and subsequent expression of inducible heat shock protein 70. *Circ Res*. 2010; 106: 102-110.
100. Qian J, Vafiadaki E, Florea SM, Singh VP, Song W, Lam CK, Wang Y, Yuan Q, Pritchard TJ, Cai W, Haghghi K, Rodriguez P, Wang HS, Sanoudou D, Fan GC, Kranias EG. Small heat shock protein 20 interacts with protein phosphatase-1 and enhances sarcoplasmic reticulum calcium cycling. *Circ Res*. 2011; 108: 1429-1438.
101. Kalmar B, Greensmith L. Induction of heat shock proteins for protection against oxidative stress. *Adv Drug Deliv Rev*. 2009; 61: 310-318.
102. Sugiyama Y, Suzuki A, Kishikawa M, Akutsu R, Hirose T, Wayne MM, Tsui SK, Yoshida S, Ohno S. Muscle develops a specific form of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. *J Biol Chem*. 2000; 275: 1095-1104.
103. Mounier N, Arrigo AP. Actin cytoskeleton and small heat shock proteins: how do they interact? *Cell Stress Chaperones*. 2002; 7: 167-176.
104. Golenhofen N, Perng MD, Quinlan RA, Drenckhahn D. Comparison of the small heat shock proteins alphaB-crystallin, MKBP, HSP25, HSP20, and cvHSP in heart and skeletal muscle. *Histochem Cell Biol*. 2004; 122: 415-425.
105. Salinthon S, Tyagi M, Gerthoffer WT. Small heat shock proteins in smooth muscle. *Pharmacol Ther*. 2008; 119: 44-54.
106. Park KJ, Gaynor RB, Kwak YT. Heat shock protein 27 association with the I kappa B kinase complex regulates tumor necrosis factor alpha-induced NF-kappa B activation. *J Biol Chem*. 2003; 278: 35272-35278.
107. Kammanadiminti SJ, Chadee K. Suppression of NF-kappaB activation by *Entamoeba histolytica* in intestinal epithelial cells is mediated by heat shock protein 27. *J Biol Chem*. 2006; 281: 26112-26120.
108. Li D, Shinagawa K, Pang L, Leung TK, Cardin S, Wang Z, Nattel S. Effects of angiotensin-converting enzyme inhibition on the development of the atrial fibrillation substrate in dogs with ventricular tachypacing-induced congestive heart failure. *Circulation*. 2001; 104: 2608-2614.
109. Cardin S, Li D, Thorin-Trescases N, Leung TK, Thorin E, Nattel S. Evolution of the atrial fibrillation substrate in experimental congestive heart failure: angiotensin-dependent and-independent pathways. *Cardiovasc Res*. 2003; 60: 315-325.
110. Chelu MG, Sarma S, Sood S, Wang S, van Oort RJ, Skapura DG, Li N, Santonastasi M, Muller FU, Schmitz W, Schotten U, Anderson ME, Valderrabano M, Dobrev D, Wehrens XH. Calmodulin kinase II-mediated sarcoplasmic reticulum Ca2+ leak promotes atrial fibrillation in mice. *J Clin Invest*. 2009; 119: 1940-1951.
111. Vest JA, Wehrens XH, Reiken SR, Lehnart SE, Dobrev D, Chandra P, Danilo P, Ravens U, Rosen MR, Marks AR. Defective cardiac ryanodine receptor regulation during atrial fibrillation. *Circulation*. 2005; 111: 2025-2032.
112. Liu J, Kam KW, Borchert GH, Kravtsov GM, Ballard HJ, Wong TM. Further study on the role of HSP70 on Ca2+ homeostasis in rat ventricular myocytes subjected to simulated ischemia. *Am J Physiol Cell Physiol*. 2006; 290: C583-91.
113. Chen Y, Kao Y, Huang C, Cheng C, Chen Y, Chen S. Heat stress responses modulate calcium regulations and electrophysiological characteristics in atrial myocytes. *J Mol Cell Cardiol*. 2010; 48: 781-788.
114. Shiroshita-Takeshita A, Schram G, Lavoie J, Nattel S. Effect of simvastatin and antioxidant vitamins on atrial fibrillation promotion by atrial-tachycardia remodeling in dogs. *Circulation*. 2004; 110: 2313-2319.
115. Shiroshita-Takeshita A, Brundel BJ, Lavoie J, Nattel S. Prednisone prevents atrial fibrillation promotion by atrial tachycardia remodeling in dogs. *Cardiovasc Res*. 2006; 69: 865-75.

116. Shiroshita-Takeshita A, Brundel BJ, Burstein B, Leung TK, Mitamura H, Ogawa S, Nattel S. Effects of simvastatin on the development of the atrial fibrillation substrate in dogs with congestive heart failure. *Cardiovasc Res.* 2007; 74: 75-84.
117. Sanchez-Quinones J, Marin F, Roldan V, Lip GY. The impact of statin use on atrial fibrillation. *QJM.* 2008; 101: 845-861.
118. Nègre-Aminou P, van Leeuwen RE, van Thiel G, Christa F, van den IJssel P, de Jong WW, Quinlan RA, Cohen LH. Differential effect of simvastatin on activation of Rac 1 vs. activation of the heat shock protein 27-mediated pathway upon oxidative stress, in human smooth muscle cells. *Biochem Pharmacol.* 2002; 64: 1483-1491.
119. Son GH, Geum D, Chung S, Park E, Lee KH, Choi S, Kim K. A protective role of 27-kDa heat shock protein in glucocorticoid-evoked apoptotic cell death of hippocampal progenitor cells. *Biochem Biophys Res Commun.* 2005; 338: 1751-1758.
120. Arrigo A. The cellular “networking” of mammalian Hsp27 and its functions in the control of protein folding, redox state and apoptosis. In: *Molecular Aspects of the Stress Response: Chaperones, Membranes and Networks.* Springer; 2007: 14-26.
121. Dobrev D, Nattel S. New antiarrhythmic drugs for treatment of atrial fibrillation. *The Lancet.* 2010; 375: 1212-1223.
122. Brundel BJ, Ke L, Dijkhuis AJ, Qi X, Shiroshita-Takeshita A, Nattel S, Henning RH, Kampinga HH. Heat shock proteins as molecular targets for intervention in atrial fibrillation. *Cardiovasc Res.* 2008; 78: 422-8.
123. Murakami M, Oketani K, Fujisaki H, Wakabayashi T, Ohgo T. Antiulcer effect of geranylgeranylacetone, a new acyclic polyisoprenoid on experimentally induced gastric and duodenal ulcers in rats. *Arzneimittelforschung.* 1981; 31: 799-804.
124. Unoshima M, Iwasaka H, Eto J, Takita-Sonoda Y, Noguchi T, Nishizono A. Antiviral effects of geranylgeranylacetone: enhancement of MxA expression and phosphorylation of PKR during influenza virus infection. *Antimicrob Agents Chemother.* 2003; 47: 2914-2921.
125. Katsuno M, Sang C, Adachi H, Minamiyama M, Waza M, Tanaka F, Doyu M, Sobue G. Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. *Proc Natl Acad Sci U S A.* 2005; 102: 16801-16806.
126. Yanaka A, Zhang S, Sato D, Tauchi M, Suzuki H, Shibahara T, Matsui H, Nakahara A, Hyodo I. Geranylgeranylacetone protects the human gastric mucosa from diclofenac-induced injury via induction of heat shock protein 70. *Digestion.* 2007; 75: 148-155.
127. Fujimura N, Jitsuiki D, Maruhashi T, Mikami S, Iwamoto Y, Kajikawa M, Chayama K, Kihara Y, Noma K, Goto C, Higashi Y. Geranylgeranylacetone, heat shock protein 90/AMP-activated protein kinase/endothelial nitric oxide synthase/nitric oxide pathway, and endothelial function in humans. *Arterioscler Thromb Vasc Biol.* 2012; 32: 153-160.
128. Hirakawa T, Rokutan K, Nikawa T, Kishi K. Geranylgeranylacetone induces heat shock proteins in cultured guinea pig gastric mucosal cells and rat gastric mucosa. *Gastroenterology.* 1996; 111: 345-357.
129. Yamanaka K, Takahashi N, Ooie T, Kaneda K, Yoshimatsu H, Saikawa T. Role of protein kinase C in geranylgeranylacetone-induced expression of heat-shock protein 72 and cardioprotection in the rat heart. *J Mol Cell Cardiol.* 2003; 35: 785-794.
130. Brundel BJ, Henning RH, Ke L, van Gelder IC, Crijns HJ, Kampinga HH. Heat shock protein upregulation protects against pacing-induced myolysis in HL-1 atrial myocytes and in human atrial fibrillation. *J Mol Cell Cardiol.* 2006; 41: 555-62.
131. Sun L, Chang J, Kirchhoff SR, Knowlton AA. Activation of HSF and selective increase in heat-shock proteins by acute dexamethasone treatment. *Am J Physiol Heart Circ Physiol.* 2000; 278: H1091-7.
132. Ooie T, Takahashi N, Saikawa T, Nawata T, Arikawa M, Yamanaka K, Hara M, Shimada T, Sakata T. Single oral dose of geranylgeranylacetone induces heat-shock protein 72 and renders protection against ischemia/reperfusion injury in rat heart. *Circulation.* 2001; 104: 1837-1843.
133. Chen H, Chien C, Yu S, Lee Y, Chen W. Cyclosporine A regulate oxidative stress-induced apoptosis in cardiomyocytes: mechanisms via ROS generation, iNOS and Hsp70. *Br J Pharmacol.* 2002; 137: 771-781.

134. Lubbers NL, Polakowski JS, Wegner CD, Burke SE, Diaz GJ, Daniell KM, Cox BF. Oral bimecromol elevates heat shock protein 70 and reduces myocardial infarct size in rats. *Eur J Pharmacol.* 2002; 435: 79-83.
135. Shinohara T, Takahashi N, Kohno H, Yamanaka K, Ooie T, Wakisaka O, Murozono Y, Taniguchi Y, Torigoe Y, Hara M, Shimada T, Saikawa T, Yoshimatsu H. Mitochondria are targets for geranylgeranylacetone-induced cardioprotection against ischemia-reperfusion in the rat heart. *Am J Physiol Heart Circ Physiol.* 2007; 293: H1892-9.