Modulators of proteostasis: therapeutic targets and diagnostic markers to halt and reverse atrial fibrillation

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Chapter 14

Summary

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Atrial fibrillation (AF) is the most common age-related cardiac arrhythmia accounting for one-third of hospitalizations for cardiac rhythm disturbances. The progressive nature of AF is rooted in sustainable structural damage in the atrial cardiomyocytes, which is already present when AF is diagnosed. Despite the efforts of electrical alleviation with the current treatment strategies, structural damage in atrial cardiomyocytes impairs electrical conduction and excitation-to-contraction coupling. Moreover, structural cardiomyocyte damage underlies the high recurrence rates after AF treatment and makes reversal to sinus rhythm in advanced stages of AF almost impossible. Obviously, there is an urgent need for improvement of AF therapies, that aim to halt and even reverse the structural damage in AF patients, as outlined in **chapter 2, 3, 4 and 5**. One possible treatment option in AF is the induction of cardio-protective heat shock proteins (HSPs). As it was previously observed for overexpressed HSPs to attenuate RhoA-induced actin stress bundle formation in HL-1 atrial cardiomyocytes, we tested whether RhoA affects HSP expression. In **chapter 6,** pathological RhoA activation was found to suppress the cardio-protective heat shock response (HSR) in HL-1 atrial cardiomyocytes by impairing the binding of heat shock factor -1 (HSF1) to the heat shock element in the promotor region of *hsp* genes. On the flip side, genetic inhibition of RhoA boosted the HSR indicating that the RhoA pathway regulates HSP expression. In **chapter 7,** we identified multiple geranylgeranylacetone (GGA) derivatives, especially GGA*-59, with improved physicochemical profiles and HSP-boosting capacities compared to the mother compound GGA. Importantly, these GGA derivatives conferred cardio-protective effects and accelerated recovery from contractile dysfunction in experimental AF. HSP boosting by treatment with GGA*-59 and recombinant HSPB1 post-tachypacing, was also found to accelerate recovery from tachypacing-induced structural remodeling in **chapter 8**. GGA*-59 increased HSPB1 levels, repressed HDAC6 activity and restored contractile protein and microtubule levels after tachypacing, indicating that HSP induction is an interesting target to accelerate recovery from AF-induced remodeling. In **chapter 9,** proof of concept for GGA treatment to induce HSP levels in the human heart was obtained. Three days of oral GGA treatment associated with higher HSPB1 and HSPA1 expression levels in right and left atrial appendages (RAA and LAA, respectively) of patients who

underwent coronary artery bypass grafting (CABG) surgery. In addition, GGA treated patients revealed more HSPB1 at the myofilaments compared to non-treated patients. These findings pave the way for further studies on the role of GGA and/or GGA-derivatives as a protective compound in experimental and clinical (post-operative) AF.

Since it is still unclear whether HSP levels can predict the stage of AF and recurrence after AF treatment, we sought to elucidate the correlation between serum and tissue HSP levels and AF in patients. In **chapter 10,** we showed that serum HSPB1, HSPA1, HSPB7 and HSPD1 levels did not correlate with the presence of AF compared to control, AF stage or AF recurrence in patients who underwent electrical cardioversion (ECV) or pulmonary vein isolation (PVI). However, HSPB1 levels were increased in serum samples of patients with an AF recurrence within one year after PVI, suggesting that HSPB1 levels may predict recurrence of AF after ablative therapy. Similar results were obtained for patients who underwent open heart surgery. In **chapter 11**, neither baseline nor follow-up serum HSP levels correlated with the presence of AF compared to control, AF stage, development of post-operative AF (PoAF) or AF recurrence. Atrial tissue (RAA and LAA) levels of HSPB1, HSPA1, HSPB5 and pHSF1 were similar between control and paroxysmal (PAF), persistent (PeAF) and longstanding persistent (LSPeAF) AF, while RAA HSPA5 levels were significantly lower in LSPeAF and HSPD1 levels higher in PeAF and in the total AF group compared to control. Both HSPA1 and HSPA5 RAA levels were higher in control patients who developed PoAF, compared to patients who did not develop PoAF. Interestingly, HSPB1 RAA levels were significantly lower and HSPA5 LAA levels higher in patients with AF recurrence after Maze surgery, on top of the treatment for their underlying heart disease compared to no AF recurrence.

In **chapter 12**, mitochondrial dysfunction was observed in tachypaced HL-1 atrial cardiomyocytes as demonstrated by increased transcription of the mitochondrial stress chaperones HSPD1 and HSPE1, fragmentation of the mitochondrial network and reduction of cellular ATP levels, mitochondrial membrane potential, mitochondrial calcium transients and maximal respiratory capacity. RAAs and LAAs from AF patients also displayed mitochondrial dysfunction, evidenced by aberrant ATP levels,

increased HSPD1 levels and fragmentation of the mitochondrial network. Tachypacing-induced mitochondrial calcium transient loss in HL-1 cardiomyocytes and decreased heart wall contractions in *Drosophilas* were prevented by the mitochondrial Ca^{2+} uniporter inhibitor Ru360. Furthermore, Ru360 treatment normalized cellular ATP levels and protected the mitochondrial network from tachypacing-induced fragmentation, but did not normalize transcription levels of HSPD1 and HSPE1 in HL-1 cardiomyocytes. These findings indicate that AF is associated with mitochondrial dysfunction and compounds directed at conservation of mitochondrial Ca^{2+} handling are protective.

In **chapter 13**, we showed that the level of cfc-mtDNA in serum is associated with AF stage, especially in male paroxysmal AF patients, and patients with recurrence of AF after PVI treatment. In addition, increased levels of cfc-mtDNA in the medium of *in vitro* tachypaced HL-1 cardiomyocytes were associated with enhanced mitochondrial damage and stress in these cardiomyocytes. Thus, AF may trigger the mtDNA release from atrial cardiomyocytes into the circulation. Future research is warranted to explore the applicability of cfc-mtDNA as a biomarker for AF.