Chapter 3

Scope of the thesis
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Modifiable cardiomyocyte targets to accelerate recovery from AF: HSPs and HDACs

AF is the most common clinical tachyarrhythmia and associated with increased cardiovascular morbidity and mortality [1, 2]. Early detection of new onset AF is essential, as the severity of AF progresses in time and the progression is associated with serious complications, such as thromboembolic events, heart failure, impaired cognitive function, increased mortality and therapy failure [2, 3]. At present, no effective curative therapy exists. Although ablative therapy initially seems promising, many patients (40-60%) have recurrences and require multiple ablation procedures [4]. Current pharmacological therapy is only moderately effective and its usage is limited by pro-arrhythmia and non-cardiovascular toxicities [5]. Electrical cardioversion is only temporarily effective and AF recurs in up to 87% of the patients [6, 7]. Thus, there is a great need to improve AF therapy.

To design new therapies, we need to understand the mechanisms driving AF progression. Various research groups discovered previously that the progressive nature of AF is rooted in AF-induced persistent structural damage in atrial cardiomyocytes, including degradation of sarcomeres (myolysis), also named structural remodeling. Structural remodeling promotes persistence of the disease [2, 8-10]. Therefore, it is important to understand molecular mechanisms driving structural remodeling with the aim to identify modifiable and druggable targets to accelerate recovery from remodeling.

As mentioned in chapter 1, studies previously demonstrated that prevention of proteostasis derailment through overexpression of heat shock proteins (HSPs) attenuates structural remodeling and thereby prevents AF progression [11-13]. In the heart, cardiomyocytes express high levels of specific members of the small HSP family, the HSPBs. These include HSPB1 (HSP27), HSPB5 (or αB-crystallin), HSPB6 (HSP20), HSPB7 (cvHSP) and HSPB8 (HSP22) are considered to safeguard cardiomyocytes from proteotoxicity by stabilizing the contractile apparatus [12-17]. Particularly HSPB1 binds to structural proteins and protects them from degradation by calpain in tachypaced atrial cardiomyocytes and human AF [9, 12, 13]. In addition, it was observed that the HSP response is temporarily activated in patients with short duration of AF, but exhausts when AF persists [9]. Consequently, cardiomyocytes lose their defense against structural changes such as myolysis, resulting in the progression of AF. In previous studies, the HSP inducer GGA was found to protect against the contractile, structural and electrical remodeling in various experimental models for AF, and consequently limit AF progression [9, 11]. Although GGA revealed protective effects, the major disadvantage of GGA is its high lipophilicity and low solubility, and therefore generally high oral dosages are required [18]. To improve the physicochemical property of GGA, 81 GGA-derivatives were synthesized and tested for their HSP inducing abilities and cardioprotective effects as described in chapter 4.

Of the 81 GGA-derivatives tested, three GGA-derivatives not only protected against tachypacing-induced contractile dysfunction in HL-1 cardiomyocytes and Drosophila, but also accelerated the recovery from contractile dysfunction in tachypaced HL-1 cardiomyocytes. Among the three derivatives, the most potent compound was GGA59, and this compound was therefore selected for further investigations as described in chapter 5.
In chapter 5, we explored the effect of the most potent GGA-derivative, GGA59, on the acceleration of recovery from tachypacing-induced contractile dysfunction in HL-1 cardiomyocytes. Furthermore, we explored the underlying mechanisms of how GGA59 aids in the recovery from tachypacing. We observed that the protective effect of GGA59 is via restoration of the microtubule network, via upregulation of the mRNA expression level of α-tubulin, resulting in the recovery of the protein expression levels of both α-tubulin and acetylated α-tubulin. Since the histone deacetylase 6 (HDAC6) initiates the disruption of microtubule network during tachypacing, as mentioned in chapter 2, the effect of GGA59 on HDAC6 activity was measured. We observed that GGA59 posttreatment suppresses HDAC6 activity in tachypaced HL-1 cardiomyocytes. In addition, transfection of recombinant HSPB1 after tachypacing also reversed contractile dysfunction, degradation of microtubule proteins, downregulation of α-tubulin gene expression level as well as reduced the HDAC6 activity in HL-1 cardiomyocytes. The beneficial effect on recovery from tachypacing by GGA59 and recombinant HSPB1 suggests that they both regulate multiple modifiable endpoints within proteostasis derailment via shielding structural and contractile proteins from calpain-induced degradation and suppression of HDAC6 activity.

In chapter 6, it was found that PARP1 is the key modulator for cardiomyocyte remodeling in various experimental model systems for AF, including tachypaced HL-1 cardiomyocyte, isolated rat adult atrial cardiomyocyte, and Drosophila. The genetic suppression of PARP1 and pharmacological inhibition by PARP1 inhibitors ABT-888 and olaparib, as well as supplementation of NAD+, protected against tachypacing-induced contractile dysfunction, via preventing the oxidative DNA damage and consequently PARP1 activation induced NAD+ depletion. These findings were further explored in patients with (longstanding) persistent AF. Patients with persistent AF revealed DNA damage and PARP1 activation, indicated by the increased PARylation levels. These findings suggest that prevention of energy loss via either inhibition of PARP1 or supplementation of NAD+ or its precursors, such as nicotinamide, is an innovative therapeutic approach to treat AF through preservation of the metabolism. Interestingly, the PARP1 inhibitor ABT-888 also accelerates recovery from tachypacing-induced contractile dysfunction in HL-1 cardiomyocytes, suggesting that modulation of this pathway may also promote recovery from clinical AF.

As mentioned in chapter 2, HDACs modulate cardiac proteostasis by deacetylating various proteins, including nuclear histones, cytosolic structural and contractile proteins, thereby inducing pathological gene expression or affecting the function of structural and contractile proteins, respectively [10, 19]. In chapter 7, we investigated the role of two classes of HDACs, class I and class IIa HDACs in the tachypacing-induced remodeling in cardiomyocytes. It was found that two HDACs, HDAC3 and HDAC5 are key modulators with converse functions in tachypaced HL-1 cardiomyocytes. Overexpression of HDAC3 revealed detrimental effects, while, overexpression of HDAC5 showed beneficial effects in both tachypaced HL-1 cardiomyocytes and Drosophila models. We found that tachypacing induces phosphorylation of HDAC5 which results in its nuclear export, and consequently activation of pathological fetal gene expression, which contributes to the cardiomyocytes remodeling and AF progression. Suppression of HDAC3 by both genetic and pharmacological approaches, and HDAC5 nuclear boosters, prevented the contractile dysfunction in both experimental models for AF. Comparable findings were observed in atrial appendages of AF patients, compared to control samples.
These findings suggest that the specific HDAC3 inhibitor and HDAC5 nuclear boosters may represent novel therapeutic candidates for the treatment of clinical AF.

In chapter 8, we summarize and discuss the data obtained in our experimental chapters and provide future perspectives related to the clinical use of heat shock protein inducers, PARP1 inhibitor, HDAC3 inhibitor and HDAC5 nuclear boosters in AF.

Reference

7. Ng KK, Skanes AC: When it comes to atrial fibrillation recurrence, perhaps we should look both left and right. The Canadian journal of cardiology 2015;31:17-19.


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