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Histidine-Rich Calcium Binding Protein: the New Regulator of Sarcoplasmic Reticulum Calcium Cycling

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Abstract

The histidine-rich calcium binding protein (HRC) is a novel regulator of sarcoplasmic reticulum (SR) Ca^{2+}-uptake, storage and release. Residing in the SR lumen, HRC binds Ca^{2+} with high capacity but low affinity. *In vitro* phosphorylation of HRC affects ryanodine affinity of the ryanodine receptor (RyR), suggesting a functional role of HRC on SR Ca^{2+}-release. Indeed, acute HRC overexpression in isolated rodent cardiomyocytes decreases Ca^{2+}-induced Ca^{2+}-release, increases SR Ca^{2+}-load, and impairs contractility. The HRC effects on RyR may be regulated by the Ca^{2+}-sensitivity of its interaction with triadin. However, HRC also affects the SR Ca^{2+}-ATPase, as shown by HRC overexpression in transgenic mouse hearts, which resulted in reduced SR Ca^{2+}-uptake rates, cardiac remodeling and hypertrophy. In fact, *in vitro* generated evidence suggests that HRC directly interacts with SR Ca^{2+}-ATPase2, supporting a dual role of HRC in Ca^{2+}-homeostasis: regulation of both SR Ca^{2+}-uptake and Ca^{2+}-release. Furthermore, HRC plays an important role in myocyte differentiation and in antiapoptotic cardioprotection against ischemia/reperfusion induced cardiac injury. Interestingly, HRC has been linked with familiar cardiac conduction disease and an HRC polymorphism was shown to associate with malignant ventricular arrhythmias in the background of idiopathic dilated cardiomyopathy. This review summarizes studies, which have established the critical role of HRC in Ca^{2+}-homeostasis, suggesting its importance in cardiac physiology and pathophysiology.

Keywords

HRC; Calcium-homeostasis; Contractility; Heart failure; Arrhythmia

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DISCLOSURES

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1. Introduction

A universal characteristic of the failing cardiomyocytes is aberrant intracellular Ca^{2+}-handling, manifested by prolonged intracellular Ca^{2+}-transients and changes in peak systolic and diastolic Ca^{2+} levels [1]. Rapid and effective regulation of the cardiomyocyte Ca^{2+} steady state is orchestrated by the coordinated function of the sarcoplasmic reticulum (SR) proteins. Therefore, targeting these molecules has been suggested to constitute promising therapy for the treatment of heart failure and malignant arrhythmias [2,3]. The histidine-rich calcium-binding protein (HRC), expressed predominantly in striated muscle [4], is a good candidate for such an approach because it is a SR component implicated in Ca^{2+}-uptake [5,6], storage [7] and release [5,8,9]. Early studies by Hofmann et al. [4], showed that HRC is expressed in the SR of rabbit skeletal and cardiac muscles. The 160 kD HRC protein stains metachromatically blue with Stainsall, a cationic dye staining positive for Ca^{2+} binding proteins and it binds $^{45}Ca^{2+}$ on nitrocellulose membranes with high capacity and low affinity [4,10]. Although it was found that this molecule could interact with low density lipoproteins, such a biological function for HRC was rejected because of its localization within the SR lumen, where it has no access to plasma lipoproteins. Instead, it was proposed that HRC may have a role in Ca^{2+}-homeostasis [4]. This notion was later supported by further experimental evidence that placed HRC at the junctional SR, which contains the ryanodine receptor channels (RyR), recognizing its involvement in excitation-contraction coupling [11–15].

The localization of HRC has been the subject of extensive debates. It was initially described as a SR lumen component [4], although Saccheto et al. [16] proposed that HRC is a cytosolic protein which interacts with the triadin cytoplasmic domain and it is phosphorylated by the SR membrane anchored calmodulin kinase II (CaMKII) on the cytoplasmic side. However, HRC is not likely to be a cytoplasmic protein, based on several lines of evidence: a) electron microscopy studies indicate a diffuse localization pattern of HRC within the terminal cisternae and the longitudinal tubules of SR [4]; b) HRC can only be released by sodium carbonate treatment of the SR, consistent with its luminal location [4]; c) HRC is present in microsomal fractions following subcellular fractionation [18]; and d) Lee et al. [8] demonstrated that HRC binds to the carboxyl-terminal luminal KEKE motif of triadin, providing further support for its SR luminal localization. In addition, several studies indicate that HRC is phosphorylated by the lumenally located casein kinase 2 (CKII) [9,15] and phosphoproteomic analysis of human skeletal muscle also confirmed that CKII is involved in HRC phosphorylation [17].

Functionally, HRC has an important role in SR Ca^{2+}-handling and in the overall cardiac physiology as it has been shown to improve the SR Ca^{2+}-storage capacity and enhance cardiomyocyte survival after ischemia [19], to alter Ca^{2+}-cycling after acute [20] and chronic overexpression [6], and to be implicated in human arrhythmia as a modifying factor [21,22].

2. Histidine-rich calcium-binding protein (HRC)

2.1. HRC gene structure and transcription

The HRC gene, located at 19q13.3 in human, and 7 B4 in mouse, consists of 6 exons, with almost 90% of the entire coding region being in its first exon [23,24]. The transcription of this gene is tissue specific, with high levels of expression in cardiac, skeletal, and to a lesser extend in arteriolar smooth muscles [23,25,26]. Three potential CpG islands could be identified at 2.1 kb, 2.5 kb, and 3.2 kb upstream of the transcriptional initiation site of HRC, but there is no identifiable TATA box. At -224 there is a GGCTGGGG sequence which is also present upstream of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase genes 1 and 2

(ATP2A1 and ATP2A2, that encode the SERCA1 and SERCA2 proteins, respectively), the fast-twitch skeletal muscle calsequestrin, dihydropyridine receptor and ryanodine receptor, suggesting that it may serve as a SR signal [27–31]. Importantly, HRC transcription during embryonic development in mouse heart is regulated by a 2.6 kb genomic region upstream of the transcriptional initiation site, that contains a small, highly conserved sequence with a consensus MEF2 binding site [32]. In contrast, the minimal genomic region, which facilitates gene expression in skeletal muscle, is 770 bp upstream of the transcriptional initiation site [32]. Since additional upstream cis-acting enhancer sequences upstream (−770) are necessary for HRC gene expression in developing cardiomyocytes, it appears that its fine regulation in human heart is of critical importance. Notably, the transcription initiation site of HRC is located within 2.3 kb from the transcription initiation site of the transient receptor potential cation channel (subfamily M, member 4, TRPM4) [33]. This Ca^{2+}-activated non-selective cation channel has been associated with human progressive familial heart block type I [34]. It appears that the HRC promoter is bidirectional regulating the transcription of both HRC and TRPM4.

The HRC protein is not detectable in undifferentiated, proliferating rat thigh muscle isolated myoblast cultures. However, HRC accumulates rapidly, when differentiation is induced [4]. This may be attributed to the SOX15-driven regulation of the HRC transcription. SOX15 is a member of the SOX (sex determining region Y-related, high mobility group-box) family of transcriptional factors, which are involved in the regulation of embryonic development, determination of cell fate and cell commitment. SOX15 antagonizes muscle differentiation in myogenic precursor cells, and regulates early myogenesis or fusion of myoblasts to myotubes [35]. SOX15 ablation in mouse embryonic stem cells results in selective overexpression of HRC mRNA, suggesting that SOX15 is a suppressor of the HRC locus [36]. Interestingly, SOX15 null mice exhibit delayed skeletal muscle regeneration after injury, although their myofiber ultrastructure is normal [37]. Similar results are obtained from cultured Sox15-deficient mouse myoblasts, which display a marked delay in differentiation *in vitro* [37]. The significance of HRC repression by SOX15 in the early stages of myocyte differentiation, especially during myofiber trauma repair, remains to be elucidated.

2.2 HRC structure and physicochemical properties

The HRC protein is a highly charged molecule with 31% acidic and 8% basic amino acid residues. There is a signal peptide at the amino-terminus of HRC (1-28 AA in human), which targets the molecule to the SR. This is followed by domains rich in: a) histidine (59-187 AA); b) glutamic acid and histidine (188-245 AA); c) aspartic acid, glutamic acid and histidine (246-309 AA); d) glutamic acid and histidine (310-468 AA); e) glutamic acid (469-608 AA); and f) cysteine (627-673 AA). An interesting feature of HRC is that it contains a highly repetitive region in the middle of the protein, between AA 106 and 365 in human, composed of ten histidine-rich acidic tandem repeats. These repeats are characterized by two types of motifs. The type A motif, which is repeated four times, starts with an almost invariant hexapeptide (HRH-R/Q-GH), followed by a stretch of acidic residues (8-18 AA in length), and ends with a conservative peptide of 9 AA (STESDRHQA). The type B motif, which is repeated six times, starts with an heptapeptide (HRHQXHG), followed by 1 to 4 amino acids, and ends with an EEDEDVSXEHHHHXPS sequence, as identified in humanHRC [23,38].

HRC lacks a typical Ca^{2+} binding motif and it is likely that the highly acidic central cores of each repeat may constitute the Ca^{2+} binding sites, in a manner similar to calsequestrin. This implies that HRC binds Ca^{2+} through electrostatic interactions with pairs or triplets of acidic amino acids, which form Ca^{2+} binding surfaces [39]. These surfaces of the ten histidine-rich acidic tandem repeats of motives A and B may be closely packed in the presence of Ca^{2+}. In

support of this notion, Ca^{2+}-binding induces changes in the electrophoretic mobility of HRC and suggests Ca^{2+}-induced conformational changes [11,15]. This is highly similar to calsequestrin and the condensed formation of its three thireodoxin-like domains in the presence of high Ca^{2+} [39,40]. Importantly, the HRC protein remains tightly attached to the SR membrane after treatment with 500 mM of potassium chloride, which removes calsequestrin. However, HRC can be readily extracted with 1 mM EDTA, suggesting that the interaction of HRC with the SR membrane components requires Ca^{2+} [14]. Picello et al. [14] reported that HRC binds 200 nmoles of Ca^{2+}/mg protein with low affinity (K_D=1.9 mM). It also binds zinc ions (Zn^{2+}) at different binding sites than Ca^{2+} while calsequestrin has no affinity for Zn^{2+} [14]. Zinc has been shown to have negative inotropic effects in the heart, causing prolonged action potentials [41]. The HRC acidic surfaces along with the C-terminal cysteine-rich domain, which is highly conserved among species, provide the means for protein-protein interactions through positively and negatively charged amino acid residues, Ca^{2+} or cysteine bridges. The lack of three-dimensional structural information on HRC limits our knowledge regarding the domains that bind Ca^{2+} and Zn^{2+}, as well as the folding changes which may be propagated by different ion saturation states.

2.3 HRC binding partners

The presence of repetitive elements, which are rich in histidine and acidic amino acids, raises the question of whether HRC could be multimeric. Indeed, Suk et al. [42] showed that purified HRC was eluted in increasing molecular size fractions under ascending Ca^{2+} concentrations. Under physiological Ca^{2+}-levels, HRC appears to be present as a multimer. However, at higher Ca^{2+}-levels and when HRC is saturated with Ca^{2+}, the protein is present as a monomer [42]. Thus, local and rapid changes of Ca^{2+}-levels within the SR lumen [43], may lead to different multimerization states of HRC and affect its interactions with other SR components.

The first SR protein identified to interact directly with HRC was triadin [8,9,16]. Triadin is embedded in the SR membrane, as part of the quaternary Ca^{2+}-release complex [40,44]. Lee et al. [8] demonstrated that HRC binds triadin's luminal KEKE motif, which is capable of interacting with calsequestrin and the ryanodine receptor [45]. Specifically, the human triadin domain, which contains a EQKKAKTAEKSEEKTKKE sequence and is similar to the EEKKARTKEKIEEKTKKE motif (KEKE-motif) of rabbit triadin, was found to interact with the human HRC [5]. It seems that multiple domains of HRC could interact with triadin, since both the C-terminal cysteine-rich domain [5,9,16] and the histidine-rich and acidic repeats [8] have been identified to target the triadin KEKE motif. Importantly, the HRC/triadin interaction is Ca^{2+} sensitive and the highest binding occurs at moderate Ca^{2+} concentrations, similar to the physiological $[Ca^{2+}]_{SR}$ [5,8,9]. As the Ca^{2+} concentration increases or decreases below that level, the HRC/triadin interaction is disrupted. Therefore, it appears that the basis of HRC/triadin interaction is electrostatic, and conformational changes of HRC due to Ca^{2+} binding, may disturb it. Binding of HRC to triadin may affect RyR function, through the quaternary (junctin, triadin, calsequestrin, and RyR) Ca^{2+}-release complex [40,44], which in turn could lead to SR Ca^{2+}-release perturbations, with detrimental effects on myocyte Ca^{2+} cycling and contractility.

Indeed, overexpression of HRC in mouse hearts decreased SR Ca^{2+}-uptake rates, without significant change of the SR Ca^{2+}-load, suggesting a suppressive role for HRC on SERCA pumping [6]. HRC was shown to bind to SERCA2a in mouse and human hearts, implicating HRC as a regulator of SR Ca^{2+}-uptake [5]. The domain of human HRC that binds to SERCA2 encompasses the second glutamic acid- and histidine-rich amino acid region. The minimal domain of SERCA required for binding to HRC contains part of the N-terminal region and is in close proximity to the cation transporter domain [46,47], which is identical in all SERCA1 and SERCA2 isoforms [48–51]. The HRC/SERCA interaction is also Ca^{2+}

sensitive, with maximal binding occurring at very low Ca^{2+} concentrations [5]. Thus, it may be hypothesized that localized reductions of SR luminal Ca^{2+} [43], promote HRC binding to SERCA. As the concentration of SR Ca^{2+} is increased, the HRC/SERCA interaction may get attenuated, resulting in dissociation of SERCA from HRC. The released HRC can then bind to triadin, and regulate Ca^{2+}-release through the quaternary RyR complex. Therefore, HRC may function as a sensor of SR Ca^{2+} changes, and may regulate both SR Ca^{2+}-release and Ca^{2+}-uptake (Fig. 1). However, future studies are needed to further investigate the reversibility of HRC/SERCA and HRC/triadin interactions *in vivo* and their functional implications on Ca^{2+}-uptake and Ca^{2+}-release, respectively.

Another important finding towards our understanding of the role of HRC in cardiac function is the identification of its interaction with a mutant variant of the junction plakoglobin, namely S39_K40insS, which has been shown to cause Naxos disease [22]. Naxos disease is an inherited disorder of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), associated with sudden cardiac death [52]. Plakoglobin is a major component of cell-cell adhesion complexes [53], the desmosomes, abundant in tissues exposed to high levels of mechanical stress, such as epidermis and cardiac muscle [54]. Although wild type plakoglobin does not bind HRC, the S39 K40insS mutant does [22]. Furthermore, bioinformatical analysis of the first 45 amino acid residues of the wild type and mutant plakoglobin, using the SignalP v. 3.0 (http://www.cbs.dtu.dk/services/SignalP/) [55], indicate that addition of an extra serine raises the probability for cleavage at position 40. Thus, mutant plakoglobin may potentially move to the SR and interact with HRC affecting Ca^{2+}-homeostasis and thereby promoting myocyte injury and/or contributing to electrical instability [22]. The role of HRC in malignant ventricular arrhythmias may be similar to calsequestrin [56–62] and RyR [63–65] human mutations, which have been suggested as causative factors of catecholaminergic or polymorphic ventricular tachycardia, and arrhythmogenetic right ventricular cardiomyopathy. The mechanism underlying these malignant arrhythmias includes impaired Ca^{2+} handling, which predisposes to delayed after depolarizations and triggered activity of arrhythmogenesis.

In conclusion, HRC binds directly to SERCA and triadin through different domains and it may regulate both SR Ca^{2+}-sequestration and Ca^{2+}-release. Thus, HRC may serve as a nodal point bridging these two key Ca^{2+}-processes in the SR. Future studies may be designed to elucidate the functional significance of these interactions under physiological and pathophysiological conditions.

2.4 Phosphorylation of HRC

In vivo phosphoproteomic studies of healthy human skeletal muscle [17] showed that HRC is phosphorylated at six serine amino acid residues, namely Ser119, Ser431, Ser563, Ser567, Ser157/Ser159 and Ser170/Ser171. In addition, the authors predicted that the majority of these sites, (Ser119, Ser170 or Ser171, Ser431 and Ser567), may be phosphorylated by the lumenally located CKII, while Ser157 or Ser159 may be phosphorylated by protein kinase B (PKB), and Ser563 by the mitogen-activated protein kinase 8 (MAPK8) or the glycogen synthase kinase 3 beta (GSK3B). This study clearly demonstrated that HRC is phosphorylated *in vivo* in healthy human skeletal muscle, but its functional significance, as well as the question for potential phosphorylation aberrations under physiological and pathophysiological conditions, remains to be elucidated. Furthermore, endogenous phosphorylation of HRC by CKII in SR membranes isolated from rabbit fast-twitch skeletal muscle has been described and associated with a decreased affinity of RyR for Ca^{2+} [13,15]. However, this observed effect on RyR activity may not be the direct outcome of HRC phosphorylation, but the result of other SR protein mediators. Given these limited data, the specific contribution of HRC phosphorylation is not clear and further studies are needed to

determine its biological significance and its potential implications on SR Ca^{2+}-uptake or release.

2.5 HRC in regulation of Ca^{2+}-homeostasis and contractility

To elucidate the functional significance of HRC, adenoviral gene transfer was used to overexpress HRC in adult rat cardiomyocytes [20]. Acute overexpression was associated with an increase of SR Ca^{2+} content, but decreased SR Ca^{2+}-release, resulting in depressed cardiomyocyte contractility. HRC overexpression was accompanied by increased levels of triadin and junctin, whereas RyR, calsequestrin, PLN and SERCA remained unaltered [20]. Importantly, a modest increase of HRC expression had a more pronounced effect on Ca^{2+} transients and cardiomyocyte contractility than a 20-fold overexpression of calsequestrin [67].

Furthermore, Gregory et al. [6] generated a transgenic mouse model with cardiac specific HRC overexpression (3-fold) to investigate and characterize the role of HRC *in vivo*. In this model, SR Ca^{2+}-uptake rates were impaired and cardiomyocyte Ca^{2+} decay was attenuated, without significant alteration in peak Ca^{2+} transient or SR Ca^{2+}-load. This phenotype was accompanied by increased triadin and NCX protein levels, as well as increased Ca^{2+} extrusion through the NCX, compared to wild types. Although the total L-type Ca^{2+}-channel (LTCC) levels were unaltered, their current density was increased, constituting a potential compensatory mechanism along with NCX upregulation to maintain SR Ca^{2+}-load in the face of depressed SR Ca^{2+} transport activity. The chronic overexpression mouse model of HRC exhibited compromised heart response to stress factors, and eventually progressed to hypertrophy and heart failure upon aging. Interestingly, the SR Ca^{2+}-uptake rates were attenuated in HRC overexpressing hearts to a similar extend as in SERCA heterozygous deficient hearts [68,69], indicating a major inhibitory effect of HRC on SERCA activity. This may be due to the binding of HRC with the SERCA domain, which is in close proximity to the cation transporter site [46,47], regulating SR Ca^{2+}-sequestration [70]. Thus, increases in the apparent stoichiometry of HRC/SERCA2 may impair SR Ca^{2+}-cycling. Indeed, the depressed Ca^{2+}-handling in human failing hearts may reflect at least partly, a relative increase in the HRC/SERCA2 levels [20,71], due to a lower reduction in HRC (17%), compared to SERCA2 (40%) levels. Such an apparent increase (1.4 fold) in the relative HRC/SERCA2 protein ratio would contribute to depressed SR Ca^{2+}-uptake and impaired Ca^{2+}-cycling in heart failure.

Interestingly, HRC overexpression was associated with significantly improved recovery of post-ischemic contractile function compared to wild type mouse hearts, while the myocardial infract size was smaller, both *ex vivo* and *in vivo* [19]. In addition, the HRC transgenic animals exhibited attenuated ischemia/reperfusion-induced apoptosis, compared to wild types. Since no significant changes were observed in SERCA, RyR, triadin, junctin and calsequestrin, before and after ischemia, a novel role of HRC in the apoptotic machinery was suggested. Indeed, the Bcl-2 protein was significantly increased following reperfusion, whereas the levels of Bax were not altered in HRC overexpressing hearts, which may favour the integrity of mitochondria, repressing mitochondrial-mediated apoptotic and necrotic death pathways. This increase of the Bcl-2/Bax ratio may be the result of the decreased free $[Ca^{2+}]_{SR}$ due to HRC overexpression, which leads to reduced intramitochondrial Ca^{2+}-accumulation. Furthermore, the active caspase-3, caspase-9 and caspase-12 were markedly decreased in transgenic hearts after ischemia/reperfusion, possibly due to increased cytosolic Ca^{2+}-levels [19]. These data suggest that inhibition of apoptotic cell death is the mechanism of protection against cardiac ischemia/reperfusion injury in HRC overexpressing animals.

Recently, an HRC knock-out mouse model was generated [72]. The HRC knock-out mice exhibited impaired weight gain, with significant reduction in muscle mass, and triadin

protein overexpression. Furthermore, this model was sensitive to isoproterenol-induced cardiac hypertrophy. Thus, alterations in HRC levels, combined with other genetic or environmental factors, may contribute to pathological hypertrophy and heart failure.

It should be noted that the majority of HRC studies in animal models predominantly focused on the role of HRC in cardiac contractility and its potential involvement in heart failure. However, since recent evidence has linked HRC to fatal arrhythmias in human heart failure patients, it becomes important to design future studies addressing the functional significance of HRC in arrhythmogenesis (see below).

2.6 Genetic variants of HRC and heart disease

Several signalling pathways are involved in the induction of pathologic remodelling and heart failure, and many of these pathways are linked to cardiac SR Ca^{2+}-cycling. The failing heart is characterized by impaired removal of cytosolic Ca^{2+}, reduced loading of the SR, and defective SR Ca^{2+}-release, culminating in impaired cardiac diastolic and systolic function [73]. Given the properties of HRC, alterations in its expression levels, function, localization, and/or regulation may disturb intracellular Ca^{2+}-homeostasis, leading to the development or altered progression of heart failure. Indeed, Fan et al. [20] reported decreased HRC protein levels in both human and experimental heart failure in mice. Decreased HRC levels were also found in a canine model of chronic heart failure while calsequestrin levels remained unaltered, compared to healthy controls [74].

In inbred mouse strains, the HRC gene has been proposed to be a candidate responsible for dystrophic cardiac calcification [75]. The working hypothesis is that aberrant expression or mutation of HRC may affect Ca^{2+}-homeostasis, resulting in increased fluctuation or average concentration of Ca^{2+} within the cell, and thus contribute to the pathology of dystrophic cardiac calcification. In humans, the HRC gene was mapped within the myotonic dystrophy associated locus, and naturally occurring genetic variants have been described [23]. Since an impairment of Ca^{2+}-handling and excitation-contraction coupling may lead to myotonia, a critical role for HRC in human cardiac disease was also suggested. Furthermore, two independent linkage analysis studies for isolated cardiac conduction and progressive familial heart block type 1 diseases, mapped the candidate gene close to the HRC locus [76,77]. Both diseases are characterized by right or left bundle branch block and QRS widening, that may progress to complete atrioventricular block with syncope and sudden cardiac death. Although the connection of isolated cardiac conduction disease with HRC is well established [76], it is not currently clear whether isolated cardiac conduction and progressive familial heart block type 1 diseases may map to the same locus [77].

Recently, a genetic variant of HRC, namely Ser96Ala, showed a statistically significant association with malignant ventricular arrhythmias and sudden cardiac death in a well-characterized cohort of patients with idiopathic dilated cardiomyopathy [21]. Kaplan-Meier survival curves during follow up of these patients indicated a markedly increased number of malignant arrhythmia episodes for the Ala/Ala homozygous patients, when compared with the Ser/Ala heterozygotes and Ser/Ser homozygotes during the first two years of the follow up study (Fig. 2). This finding was independent of medication and the patients' clinical characteristics, such as atrial fibrillation or left bundle branch block. The mechanism may be associated with impaired phosphorylation at Ser96 by casein kinase II. The abolishment of the phosphorylation site could affect HRC interactions with SERCA2 and/or triadin [5], resulting in RyR destabilization and exacerbation of spontaneous Ca^{2+}-release and delayed after depolarizations, leading to lethal arrhythmias in idiopathic dilated cardiomyopathy. Future studies in isolated cardiomyocytes and transgenic mouse models will further explore the underlying mechanism of ventricular arrhythmia susceptibility in Ala/Ala homozygous dilated cardiomyopathy patients.

3. Conclusions and future perspective

Increasing evidence suggests that the impaired function of the SR, consisting of Ca^{2+}-uptake, storage, and release, is a critical characteristic of failing hearts. HRC holds an important role in cardiomyocyte physiology, mediating a fine cross-talk between SR Ca^{2+}-uptake and release, through its direct interactions with SERCA and triadin. Thus, HRC may act as an intra-SR Ca^{2+}-sensor that mediates different responses upon luminal SR Ca^{2+}-fluctuations. Although HRC overexpression is associated with cardiac dysfunction, the protein appears to have a cardioprotective effect after ischemic episodes. The recent identification of the Ser96Ala HRC genetic variant as an independent predictor of susceptibility to arrhythmogenesis in the setting of dilated cardiomyopathy, points to the importance of HRC in cardiac function that merits careful consideration and further analysis under physiological and pathophysiological conditions.

Future studies need to explore the HRC role in myocytes at multiple levels. Biophysical analysis of the three-dimensional structure of HRC protein under different Ca^{2+} and phosphorylation conditions will allow the identification of altered folding patterns and interactions with protein partners. Furthermore, it would be interesting to explore whether the HRC/SERCA and HRC/triadin interactions are reversible *in vivo*, under different Ca-luminal conditions. In addition, investigation of the HRC transcriptional regulation during myocyte differentiation and heart disease may reveal important insights in its functional significance. Finally, exploring the mechanisms underlying the Ser96Ala HRC genetic variants and the susceptibility to malignant arrhythmias may provide new insights to pathways of excitation-contraction coupling and action potential control, leading to the development of novel clinical interventions.

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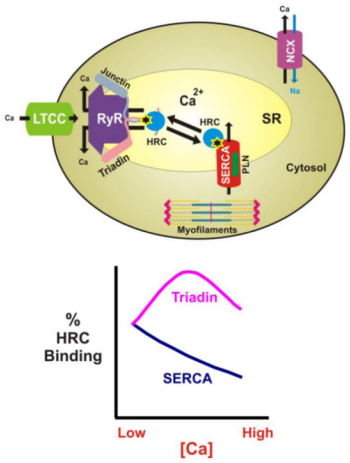

Fig. 1.

Schematic representation of HRC interactions within the SR. The interactions are indicated with a star. HRC could directly bind either the RyR quaternary complex partner triadin or SERCA (upper panel). These interactions are Ca^{2+}-sensitive (lower panel) and HRC may function as a sensor of intra-SR Ca^{2+}-changes, regulating SR Ca^{2+}-release and/or uptake.

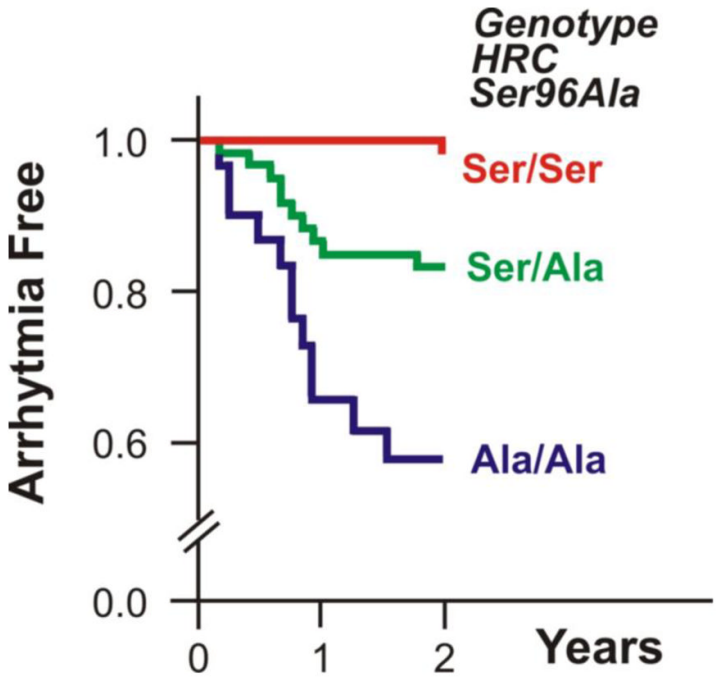

Fig. 2.

Two year Kaplan-Meier plots for the probability of survival of idiopathic dilated cardiomyopathy (DCM) patients from life-threatening ventricular arrhythmic events, defined as sudden cardiac death, episodes of unstable ventricular tachycardia or ventricular fibrillation. The Ala/Ala-HRC homozygous dilated cardiomyopathy patients were more susceptible to ventricular arrhythmic events, compared with the Ser/Ala-HRC heterozygotes and Ser/Ser-HRC homozygotes.