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EDITORIAL COMMENT

Cardiac BIN1 Replacement Therapy Ameliorates Inotropy and Lusitropy in Heart Failure by Regulating Calcium Handling*

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Bridging integrator 1 (BIN1) is a scaffold protein belonging to the superfamily of proteins containing the BAR domain, first identified by its common occurrence in vertebrate Bin1 and Amphiphysins and in the *Saccharomyces cerevisiae* Rvs proteins. Specifically, BIN1 has its BAR domain within its N-terminus, encoded by exons 1 to 9. Architecturally, BIN1 forms a homodimer through the interaction between helixes of the BAR domain from each monomer; such interaction produces a peculiar spatial disposition with a 6-helix bundle formation, conferring an elongated banana shape to BIN1 dimers. The *BIN1* gene is composed of 20 exons, the alternative splicing of which generates tissue-specific BIN1 isoforms with different functions. Among more than 10 isoforms, there are 2 cardiac-specific spliced variants: BIN1+13 and Bin1+13+17, also known as cardiac-BIN1 (cBIN1). BIN1+13 is the most abundant isoform in the heart and seems to be implicated in cell proliferation. cBIN1 is the isoform localized on cardiac transverse tubules (T-tubules), where it is indispensable in packaging T-tubule membrane microfolds (1), exploiting its membrane-curving abilities. In addition to enabling correct invagination of the T-tubules, cBIN-1 mediates the formation of functional complexes between the L-type calcium channel (LTCC, also known as dihydropyridine channel) and the intracellular calcium release channels ryanodine receptor type 2 (RyR2); these functional complexes are known as LTCC-RyR2 dyads. As a direct consequence, cBIN1 has a pivotal role in ensuring a proper contraction of cardiomyocytes and has been proposed as a potential therapeutic target in heart failure (HF). Indeed, levels of cBIN1 have been shown to be reduced in animal models of HF, as well as in human biopsy samples from patients with end-stage cardiomyopathy.

In this issue of *JACC: Basic to Translational Science*, Liu et al. (2) evaluate the effects of cBIN1 replacement therapy in murine HF models obtained by chronic exposure to isoproterenol (ISO) or by pressure overload (transverse aortic constriction [TAC]). In both the ISO and TAC models of HF, normalization of cBIN1 levels through adeno-associated virus 9 (AAV9)-mediated gene therapy was shown to effectively restore T-tubule/c-BIN1 microfolds and the distribution of calcium regulatory proteins. This molecular remodeling was associated with an improvement of systolic and diastolic function in the ISO and TAC models. A key finding is that only BIN1-13+17 (cBIN1)

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administration, rather than other isoforms of cBIN, increases LTCC localization to T-tubules and affects T-tubule remodeling. An additional novel finding in the study by Liu et al. is that exogenous cBIN1 increases SERCA2a function through organizing its intracellular distribution, although this was shown to be not specific for the cBIN1 isoform. Before discussing this paper further, it is useful to digress for a moment and review the role of cBIN and calcium handling in the normal and failing heart.

cBIN1 AND CARDIAC CALCIUM HANDLING

After the depolarization of the sarcolemma membrane, a relatively small amount of calcium enters into cardiomyocytes via the LTCC. Calcium entry then elicits a massive calcium release from the sarcoplasmic reticulum (SR) via RyR2 channels (3,4). This mechanism, referred to as calcium-induced calcium release, activates the contractile machinery (3). The efficacy of calcium release from the SR essentially depends on the proximity of LTCC channels on the plasma membranes and RyR2 channels on the SR. This feature is one of the main reasons why the plasma membrane of cardiac cells is organized in T-tubules, membrane invaginations that ensure a precise closeness between LTCC and RyR2 channels. Liu et al. (2) did not actually measure the effects of cBIN1 replacement on SR calcium load, calcium leak, or calcium reuptake. Nevertheless, cBIN1 seems to structurally and functionally contribute to LTCC and RyR2 channel coupling, at least via 3 mechanisms: 1) through its banana-like shape, cBIN1 produces the fitting membrane invagination in the T-tubule; 2) cBIN1 can act as an anchor protein for LTCCs, regulating their trafficking and clustering at the dyad level; 3) cBIN1 recruits the other major dyadic protein, RyR2, creating the perfect molecular bridge between 2 calcium channels on the 2 compartments, plasma membrane and SR (3).

Another important aspect is that the cBIN1-mediated bridge is not a static assemblage; rather, it is a dynamic spatial and temporal organization capable of responding to stress, for example, by supporting the increased cardiac output demand. Indeed, the recruitment of RyR2 by cBIN1 is phosphorylation dependent: this selective action on Ser^{2808}-phosphorylated RyR2 allows the dyads to include the channels with increased calcium sensitivity and open probability, thereby supporting the higher cardiac demand during stress (4). Furthermore, the cBIN1 dyad complexes can be rapidly arranged in response to different stimuli. For instance, the activation of β-adrenergic receptors by catecholamines during stress is known to induce a rapid cBIN1 redistribution, probably acting on the accumulation of BIN1-binding phospholipids. Given its crucial role in ensuring the structural and electrical stability of cardiac dyads, it is not surprising that a compromised cBIN1 function contributes to contractile pump failure and/or arrhythmias, further underscoring the fundamental importance of this molecule both as a disease marker and therapeutic target.

cBIN1 IN THE PATHOGENESIS OF HF AND ARRHYTHMIAS

T-tubule remodeling with the consequent dyad uncoupling and altered cardiac calcium transients is a well-established hallmark of HF development and progression (1,3). The alterations of cBIN1 observed in HF provide another proof of concept of the functional contribution of T-tubule dysregulation to HF pathophysiology. The first evidence supporting the role of cBIN1 in HF came from the observation of a significant down-regulation of BIN1 in human failing cardiomyocytes, both at the messenger ribonucleic acid and protein levels; BIN1 knockdown in murine and zebrafish cardiomyocytes triggers a significant impairment in calcium transients and a severe contractile dysfunction. These findings prompted similar research in other animal models, confirming decreased cBIN1 and T-tubule density in sheep models of HF induced by right ventricular tachypacing or ascending aortic coarctation. In both models, the alterations in cBIN1 and T-tubule density correlate with amplitude reduction and higher heterogeneity of systolic calcium transients.

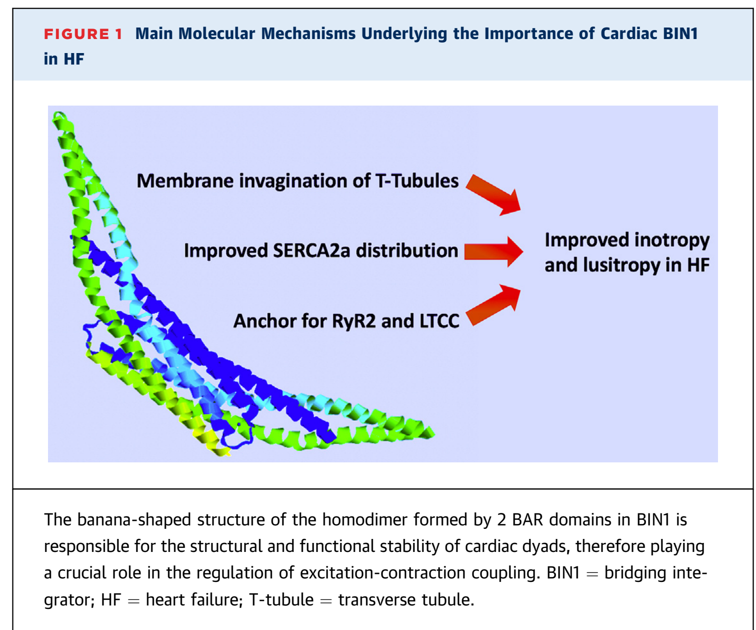
Whereas the evidence described comes mostly from observational studies, what happens when BIN1 is actively removed in the heart? Cardiac-specific ablation of the *BIN1* gene is sufficient to generate per se a model of HF in aged mice, causing dilated cardiomyopathy with an approximately 45% reduction in ejection fraction beginning at approximately 8 to 10 months of age. Interestingly, although younger animals are protected from HF, they rapidly develop a frank dilated cardiomyopathy in response to pressure overload. At the molecular level, Bin1 cardiac deficiency induces mislocalization of voltage-dependent calcium channels Cav1.2 and interstitial fibrosis. Additionally, the gene encoding for BIN1 seems to be haploinsufficient, as heterozygous mice exhibit an intermediate phenotype. The loss of cBIN1 strongly affects electrical coupling in cardiomyocytes, increasing the risk of arrhythmogenesis. Actually, when cBIN1 is not available, the formation of leaky

RyR2 clusters occurs outside the dyads, leading to arrhythmias (1). Moreover, the reduction of cBIN1 as occurs in cardiomyopathies leads to free diffusion of local extracellular calcium and potassium ions, thereby prolonging the action potential duration and increasing susceptibility to cardiac arrhythmias.

As mentioned, cBIN1 levels seem to reflect cardiac calcium-induced calcium release efficiency and the resultant pumping capacity of the heart. The pathogenic mechanisms that emerged in preclinical models have been recently corroborated in humans, demonstrating that reduced plasma BIN1 levels can predict the incidence of arrhythmias, making cBIN1 a novel and promising biomarker of cardiac (dys)function. Nikolova et al. (5) developed a cBIN1 score based on circulating levels of cBIN1 detected in HF with preserved ejection fraction (HFpEF), healthy control participants, and a control participant who had risk factors for developing HF. The cBIN1 score was computed as the natural log of the inverse of measured cBIN1 plasma concentration. The authors reported that the cBIN1 score was similar among healthy participants, as well as among patients with risk factors but not HF, whereas the score is significantly higher in HFpEF patients. Kaplan-Meier analysis of 1-year cardiovascular hospitalizations adjusted for age, sex, body mass index, and N-terminal pro-B-type natriuretic peptide levels showed that patients with HFpEF with a cBIN1 score ≥1.80 had a 3.8-fold greater risk for hospitalizations compared with those with scores of <1.80. In this context, cBIN1 not only has a predictive value for HFpEF diagnosis but also a prognostic value for the hospitalization of patients during the first year of follow-up.

The report by Liu et al. (2), extends our knowledge with respect to the molecular mechanisms (**Figure 1**) linking cBIN1 to left ventricular hypertrophy and diastolic dysfunction. Whether the murine models used in this study are relevant to patients with HFpEF is unknown and will require further validation in different models of HFpEF. Nonetheless, these studies raise the interesting possibility that normalizing myocardial cBIN1 levels may improve outcomes in HF with a reduced and preserved EF.

FIGURE 1 Main Molecular Mechanisms Underlying the Importance of Cardiac BIN1 in HF



The banana-shaped structure of the homodimer formed by 2 BAR domains in BIN1 is responsible for the structural and functional stability of cardiac dyads, therefore playing a crucial role in the regulation of excitation-contraction coupling. BIN1 = bridging integrator; HF = heart failure; T-tubule = transverse tubule.

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