Unc'ing Ca²⁺, SERCA and cardiac disease.

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Abstract

Loss of SERCA function contributes to reduced contractility, Ca²⁺ overload and arrhythmias in the diseased heart. A long non-coding RNA (*ZFAS1*) upregulated in cardiac disease is reported to directly inhibit SERCA function. The implications for cardiac disease and the wider roles of SERCA are discussed.

Introduction

***ZFAS1* and SERCA regulation in cardiac disease**

Increases in cytosolic [Ca²⁺]_i induce cardiac myocyte (CM) contraction underlying the pumping action of the heart. While Ca²⁺ influx via L-type voltage Ca²⁺ channels is an initial event in coupling plasma membrane depolarization and contraction, the Ca²⁺ release from the sarcoplasmic reticulum (SR) intracellular Ca²⁺ store that it triggers predominantly contributes to the Ca²⁺ transient. To induce relaxation, cytosolic Ca²⁺ is returned to baseline levels primarily by sequestration into the SR by the SR Ca²⁺ ATPase (SERCA), which accounts for removal of 70 to 90% (species dependent) of the cytosolic Ca²⁺. Supporting the importance of SR Ca²⁺ homeostasis and SERCA activity in the heart, reductions in SR Ca²⁺ load, Ca²⁺ transient amplitude and SERCA function are common features of diseased CM, whereas increases in these parameters mediate enhanced cardiac output during periods of increased demands, such as the fight or flight response (Figure 1) [1, 2].

Of the 3 SERCA isoforms, SERCA2a (ATP2A2) is primarily expressed in the heart, although SERCA2b, a splice variant with an extended COOH-terminus is also expressed. SERCA isoforms are regulated by cytosolic and SR/ER luminal Ca²⁺ and show isoform specific kinetics (Figure 1). In the heart, SERCA2a exhibits greater activity than SERCA2b, which makes it better suited to rapidly deal with the large cyclical changes in [Ca²⁺]_i. SERCA is also regulated by the 52 amino-acid protein phospholamban (PLB) in the heart (also sarcolipin in atria). Non-phosphorylated PLB, interacts with and suppresses SERCA activity whereas phosphorylation by PKA or CaMKII leads to PLB dissociation and SERCA disinhibition [3] (Figure 1). These phosphorylation events substantially underlie the inotropic (increased contraction) and lusitropic (increased relaxation) responses during periods of increased demands.

A new class of micropeptide modulators that directly interact with SERCA have recently been discovered. The 34 amino acid micropeptide DWORF was found to be encoded by a small functional open reading frame in a previously annotated non coding RNA [4]. DWORF associates with SERCA2a via the same region as PLB, counteracting its inhibitory function. Consistent with reduced SERCA function in disease, DWORF was downregulated under pathological conditions. SERCA is regulated through interactions with a number of other

proteins and by post translational modification such as phosphorylation and nitrosylation, allowing crosstalk with diverse signalling pathways [3].

The nodal contribution of SERCA2a to cardiac pathophysiology has led to its targeting for therapeutic benefit. Motivated by rescue of diminished Ca^{2+} transients and contraction associated with disease in cellular and animal studies, a clinical trial in which SERCA was overexpressed via an adeno associated viral vector (AAV) was initiated. Although initial results were promising, a more extensive study showed no benefit. It appears that insufficient AAV-mediated SERCA expression caused the failure of this study [5]. Given the difficulties with viral approaches to modulate protein expression in vivo, small molecules that act to enhance endogenous SERCA or prevent suppression by PLB may represent an alternative approach [6]. Indeed, increasing cytosolic Ca^{2+} clearance and SR Ca^{2+} load through SERCA activation by ochratoxin A or jasmonate or by using a PLB antibody Fab fragment raises the threshold for induction of Ca^{2+} waves, which are considered potentially arrhythmogenic [7].

Regulation of SERCA by a long non-coding (lnc)RNA called *ZFAS1* was recently described (Figure 1) [8]. LncRNAs are classified as non-coding transcripts (no open reading frame) of 200 nucleotides or more. Over 23,000 lncRNAs are predicted in humans and 11,000 in mice [9]. By comparison, ~25,000 protein coding genes are annotated in the human genome. Notably up to 90% of the genome is transcribed to RNA, of which only ~2% is protein coding, raising the possibility for discovery of an even greater number of functionally important ncRNA molecules. LncRNAs participate in many cell processes essential for life and contribute to pathology, including in cardiac disease [10]. Their mechanisms of action include acting as a scaffold for transcriptional regulators, a decoy for microRNAs and direct (*cis*) or indirect (*trans*) post-transcriptional or post-translational processing of RNAs.

ZFAS1 was reported to suppress the ATPase function of SERCA through a direct RNA-protein interaction (Figure 1) [8]. The upregulation *ZFAS1* in the diseased heart (myocardial infarction in mice and human) suggests that it could contribute to the associated decreased SERCA function. The substantial reduction in SERCA expression following *ZFAS1* overexpression may however imply that this lncRNA also acts through a siRNA or miRNA mechanism. While lncRNAs have previously been shown to act via protein interactions and to control gene expression, the direct effect of *ZFAS1* on SERCA function is however a first in terms of lncRNA regulation of Ca^{2+} handling protein activity. The possibility for regulation of this *ZFAS1*/SERCA interaction by other SERCA interacting proteins or post-translational modifications will be interesting to explore. Moreover, given the extent of the ncRNA repertoire, the potential for a new toolkit of RNA-based regulators of Ca^{2+} handling proteins is great.

Non-cardiac functions of *ZFAS1* SERCA interactions.

The substantial homology between SERCA2a and b would make it likely that *ZFAS1* also regulates this ubiquitously expressed SERCA isoform. *ZFAS1* is expressed in non-cardiac tissues and is upregulated in certain cancers, including prostate cancer. Given that ER Ca^{2+} levels play important roles in cell signalling, metabolism and regulating cell viability, the *ZFAS1*/SERCA2 interaction likely plays important roles in many cell processes and diseases. For example, ER Ca^{2+} levels are a determinant of Ca^{2+} release through InsP_3Rs , which in turn impacts downstream processes [11]. Under physiological conditions, small Ca^{2+} transfers from the ER to the mitochondria stimulate energy production and promote cell survival, whereas, excessive Ca^{2+} transfer from the ER to mitochondria leads to mitochondrial Ca^{2+} overload inducing apoptosis [12]. Reducing SERCA activity or expression, causing a reduction in ER

store content, reduces Ca^{2+} transfer from ER to mitochondria, thereby protecting against cell death. Optimal ER Ca^{2+} levels are also essential for folding and/or trafficking of proteins that reside or transit through the secretory pathway [13]. Chronic or substantial reduction in store content hampers protein folding triggering ER stress, which if not alleviated results in cell death.

Based on these known mechanisms of action, it comes as no surprise that SERCA regulation is altered in cancer to promote cell survival over cell death [13]. Whether *ZFAS1* suppression of SERCA participates in regulating cell viability in cancer remains to be established. The potential for *ZFAS1* regulation of SERCA in cancer is supported by the actions of oncogenes and tumour suppressors on SERCA function. The tumour suppressor p53 interacts with and stimulates SERCA activity, thereby increasing ER Ca^{2+} store content, promoting pro-apoptotic Ca^{2+} transfer to the mitochondria [12]. In cancer, p53 loss of function is common and allows cells to evade this manner of cell death induction. Cancer cells often upregulate Bcl-2, which targets several SERCA isoforms [12]. Bcl-2 is reported to inhibit or stimulate SERCA [12]. While it is surprising that some cancer cells increase while others decrease SERCA function both actions likely can have benefit. Reducing SERCA activity or expression lowers the ER-store content thus lowering pro-apoptotic Ca^{2+} signalling events whereas increasing SERCA allows more rapid removal of pro-apoptotic Ca^{2+} signals from the cytosol promoting survival. Despite increased SERCA activity and ER Ca^{2+} , fewer ER-mitochondrial contact sites may also serve to reduce pro-apoptotic ER-mitochondrial Ca^{2+} flux [14].

Conclusion

The deconvolving of the non-coding transcriptome will undoubtedly lead to the discovery of new RNA-based regulators of the Ca^{2+} handling toolkit. The ability to target these structural or sequence-based RNA protein interactions will pave the way for a new generation of therapies.

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Competing interests:

None to declare

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Decreased SR Ca^{2+} and SERCA activity contribute to cardiac dysfunction in disease.

The *ZFAS1* long non-coding RNA is upregulated in cardiac disease.

ZFAS1 is reported to directly inhibit SERCA activity.

By homology with *SERCA2a*, *ZFAS1* may also regulate the ubiquitous *SERCA2b* isoform.

Potential *ZFAS1* roles in pathologies involving dysregulated ER Ca^{2+} are discussed.

Healthy

Diseased

Increased ZFAS1, decreased SERCA and SR Ca²⁺
Pathological Ca²⁺ handling
Decreased cardiac function

Figure Caption

Model for the impact of SERCA regulation by the LncRNA *ZFAS1* on Ca^{2+} handling and contraction in the diseased cardiac myocyte.