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Signalosomes as Therapeutic Targets

Alejandra Negro, Ph.D.¹, Kimberly Dodge-Kafka, Ph.D.², and Michael S. Kapiloff, M.D.Ph.D.¹

¹*Cardiac Signal Transduction and Cellular Biology Laboratory, Department of Medicine and Pediatrics, University of Miami Miller School of Medicine, R198, P.O. Box 016960, Miami, FL 33101*

²*Calhoun Center for Cardiology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030*

Abstract

Cardiac hypertrophy is the predominant compensatory response of the heart to a wide variety of biomechanical stressors, including exercise, hypertension, myocardial infarction, intrinsic cardiomyopathy or congenital heart disease. Although cardiac hypertrophy can maintain cardiac output in response to elevated wall stress, sustained cardiac hypertrophy is often accompanied by maladaptive remodeling which can ultimately lead to heart failure. Cultured cardiac myocytes, transgenic and knock-out animal models, and pharmacological studies have not only revealed key molecules involved in hypertrophic signaling, but have also highlighted the redundancy in the hypertrophic signaling cascade. Currently, the majority of existing therapies for inhibition of pathologic cardiac hypertrophy and heart failure target molecules on the surface of cardiac myocytes, such as G-protein coupled receptors (GPCRs) and ion channels. Because these molecules are upstream of multiple intracellular signaling pathways, however, current therapy is often accompanied by significant off-target effects and toxicity. More recently, research has focused on identifying the intracellular effectors of these signaling cascades in the hope that more selective drugs may be rationally designed for therapeutic intervention.

Within the cardiac myocyte, the formation of discrete multimolecular complexes, or ‘signalosomes’, is an important mechanism for increasing the specificity and efficiency of hypertrophic signal transduction. In response to extracellular stimuli, these signalosomes can alter gene and protein expression, cell size, and chamber remodeling, such as in the case of the signalosomes formed by the mAKAP β and AKAP-lbc scaffold proteins. A better understanding of the basic molecular mechanisms regulating the compartmentation and scaffolding of signaling molecules could lead to the development of new clinical tools that may prevent the development of heart failure and minimize negative impacts on physiological processes.

Keywords

signalosome; mAKAP; AKAP-lbc; heart; therapeutic

Address for correspondence: Michael S. Kapiloff, Cardiac Signal Transduction and Cellular Biology Laboratory, University of Miami Miller School of Medicine, R198, P.O. Box 016960, Miami, FL 33101; email: mkapiloff@med.miami.edu, phone: 305-243-7863, fax: 305-243-3906.

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Introduction

Cellular processes such as cardiac myocyte contractility and hypertrophy are regulated by a complex intracellular network of signal transduction pathways [1,2]. These signaling pathways are composed of cell surface receptors, second messengers, ion channels, protein kinases and other enzymes, and effector proteins such as transcription factors and translational regulators. Many individual signaling components participate in multiple signaling pathways and contribute to the spatial and temporal regulation of diverse cellular processes. The utilization of the same molecules for different purposes is both an efficient use of cellular resources and a simple mechanism to permit crosstalk between different pathways. Pleiotropism, however, presents a theoretical problem for specificity in signaling. It has become appreciated in recent years that several mechanisms exist in cells to retain signaling specificity while permitting pleiotropism, including, prominently, the physical separation and spatial compartmentation of different pathways[3,4]. Signaling compartmentation is conferred in part by the formation of “signalosomes,” multimolecular protein complexes composed of unique combinations of signaling pathway components that are targeted in discrete intracellular locations via their association with anchoring or adaptor proteins. The co-localization of pathway components permits the efficient transmission of signals between adjacent molecules while avoiding inappropriate cross-talk with other pathways in the cells. As our understanding of these signalosomes grows, we should be able to identify novel specific targets for therapeutic intervention. For example, if we can uniquely affect an individual signalosome with a therapeutic agent, we may be able to modulate only the germane cellular function, without interfering in other important processes. In this review, we will discuss two different examples of signalosomes that are present in the myocyte and that are likely regulators of cardiac hypertrophy. These signalosomes are formed by the scaffold proteins mAKAP β and AKAP-lbc[5,6]. Potential specific interventions will be considered that may target these signalosomes.

Conventional Therapy

Most currently prescribed drugs that affect the heart directly are agonists or antagonists for a particular regulatory molecular or receptor [7]. For example, dopamine, dobutamine, epinephrine, phenylephrine, and norepinephrine activate adrenergic receptors, while atenolol, esmolol, and propranolol are all β -receptor blockers. Many of the anti-arrhythmic agents are ion channel blockers, such as disopyramide, flecainide, lidocaine, procainamide, and verapamil. Digoxin, a mainstay of heart failure therapy, is a Na^+/K^+ pump inhibitor. Two drugs that are directed at intracellular targets are the phosphodiesterase inhibitors amrinone and milrinone. While each of these drugs has its indications, all are accompanied by significant side effects. Because they act against all instances of the target molecules, all processes that involve that particular target are affected. For example, β -receptors regulate not only the level of myocyte ionotropy, lusitropy, and chronotropy, but also myocyte apoptosis, gene expression, and hypertrophy. While prolonged stimulation or antagonism may have beneficial effects for contractility or hypertrophy, prolonged β -stimulation is also accompanied by the stimulation of cardiac processes that are detrimental. In theory, it would be ideal if it were possible to stimulate contractility, while not inducing apoptosis or pathologic hypertrophy. Inhibition of ion channels and phosphodiesterases are similarly problematic, between these molecules are involved in the regulation of ions and cAMP that regulate many intracellular processes.

Compartmentation

About 25 years ago, it was recognized that separate pools of the cAMP-dependent protein kinase (PKA) were activated differentially by β -agonists and prostanoids [3]. PKA holoenzyme in the heart could be divided into particulate and soluble pools [8]. Later, it was discovered

that the particulate pools of PKA were the result of PKA anchoring at cytoskeletal and membranous sites by A-kinase anchoring proteins (AKAPs) [9]. AKAPs are a functional diverse family of proteins that share in common a 18 amino acid residue binding site for the regulatory subunit dimer of the PKA holoenzyme. In addition, each AKAP is located at a distinct intracellular location near PKA substrates through a unique targeting domain. For example, in ventricular myocytes the 81 amino acid residue protein AKAP18/15 is targeted to the plasma membrane through N-terminal myristoylation and palmitoylation where it may bind and enhance the phosphorylation of the L-type calcium channel [10]. By co-localizing PKA and calcium channel, AKAP18/15 specifically increases the efficiency of catecholamine-stimulated channel activation and ionotropy [11].

mAKAP β

Containing 2314 amino acid residues, mAKAP β (muscle-AKAP type β) is a much larger protein than AKAP18/15 and serves as the scaffold for a multimolecular signalosome [5]. mAKAP β was initially identified by expression cloning as a PKA binding protein in 1995 [12]. Although early studies suggested that mAKAP β may be more broadly localized [13,14], definitive studies have confirmed that mAKAP β is primarily located at the cardiac myocyte nuclear envelope [15–17]. mAKAP β is targeted to the nuclear envelope by the direct binding of spectrin-repeat domains in mAKAP β and nesprin-1 α , an integral membrane protein specific for the nuclear envelope [17]. Nesprin-1 is a KASH domain protein that is bound to the nuclear lamina through interactions with emerin, lamin A/C, and SUN-domain proteins. Interestingly, mutations in the gene for nesprin-1, like emerin and lamin A/C, have been implicated in the pathogenesis of Emery-Dreifuss muscular dystrophy (EDMD) [18]. EDMD is a slowly-progressing muscle wasting disease and cardiomyopathy that usually presents with heart block. Because nesprin-1 is mislocalized in Emery-Dreifuss muscular dystrophy, it is reasonable to speculate that mAKAP β -related signaling may contribute to the pathologic phenotype.

While it has been understood that PKA could be tethered to intracellular domains by AKAPs, it remained an open question how cAMP itself might be compartmentalized. In 2001, we showed for the first time that an AKAP could also bind a phosphodiesterase [19]. By binding phosphodiesterases, AKAPs can regulate local cAMP and prevent inappropriate PKA activation [20]. mAKAP β binds directly type 4D3 phosphodiesterase (PDE4D3) [19]. PDE4D3 is a PKA substrate and PKA phosphorylation increases both the binding affinity of PDE4D3 for mAKAP β and the PDE4D3 phosphodiesterase activity [19,21]. As a result, cAMP, PKA, and PDE4D3 form a negative feedback loop. cAMP activates PKA, which in turn activates PDE4D3, causing increased cAMP degradation. Thus, PDE4D3 serves to modulate PKA activation within the mAKAP β signalosome.

mAKAP β -associated PDE4D3 also binds ERK5 mitogen-activated protein-kinase [22]. ERK5 is thought to be important in the cardiac myocyte for promoting cell survival and hypertrophy in the face of cardiac stress [23,24]. ERK5 phosphorylates PDE4D3 on a separate residue than PKA, inhibiting the phosphodiesterase [25]. As a result, ERK5 activation results in increased local cAMP levels, potentiating PKA activation [22]. While ERK5 and cAMP synergistically activate PKA, cAMP inhibits ERK5 activation, in this case through a rap1-dependent pathway. cAMP stimulation will activate rap1 resulting in the inhibition of ERK5 and the decreased inhibition of PDE4D3, ultimately opposing cAMP accumulation. Thus, the mAKAP β signalosome contains a second negative feedback loop controlling local cAMP levels. In myocytes, ERK5 is activated by IL-6 type cytokines and by α -adrenergic agonists [24]. Because IL-6 type cytokines are secreted by cardiac fibroblasts during prolonged periods of stress, [26], one might predict that catecholamines and cytokines will synergistically activate mAKAP β -associated PKA in myocytes. By containing two negative feedback loops, the mAKAP β signalosome is poised to regulate finely local cAMP levels.

How might mAKAP β -bound PKA and ERK5 contribute to cellular regulation? A small pool of ryanodine receptors (RyR2) are associated with mAKAP β at the cardiac myocyte nuclear envelope [16,17]. RyR2 are calcium-stimulated calcium release channels that are mainly responsible for the release of bulk calcium from the sarcoplasmic reticulum during excitation-contraction coupling. Although the mechanism is somewhat controversial, it is generally accepted that RyR2 currents can be increased by PKA phosphorylation [27,28]. There are also RyR2s at the nuclear envelope. The nuclear envelope is composed of the inner and outer nuclear membranes. The intervening perinuclear space contains stores of calcium ion that are in equilibrium with sarcoplasmic stores [29]. β -agonist stimulation will result in PKA-phosphorylation of mAKAP β -associated RyR2 and presumably increased local calcium levels [16]. mAKAP β also binds calcineurin A β , the calcium/calmodulin-dependant phosphatase required for the induction of cardiac hypertrophy and that regulates NFATc transcription factor activation [30,31]. In fact, we have found that mAKAP β expression is required for the full-activation of NFATc transcription factor in myocytes and for the induction of myocyte hypertrophy by adrenergic agonists [30].

While PKA may phosphorylate RyR2, contributing to calcineurin A β stimulation, the function of mAKAP β -associated ERK5 is less clear. Interestingly, mAKAP β expression is also required for the induction of myocyte hypertrophy by the IL-6 type cytokine leukemia inhibitory factor [22]. In sum, by its location at the nuclear envelope, the mAKAP β signalosome may be strategically located to regulate pro-hypertrophic and anti-apoptotic signal transduction where it may serve to integrate both ERK5 and cAMP signaling (Figure 1).

AKAP-lbc

AKAP-lbc is a second scaffold that has been implicated in the regulation of cardiac myocyte hypertrophy [32]. A fragment of AKAP-lbc called Ht31 (human thyroid clone 31) was initially cloned like mAKAP β through its ability to bind PKA [33]. In fact, the Ht31 PKA-binding peptide has been used extensively in functional studies demonstrating PKA anchoring in cells, and the structure of Ht31 peptide binding to PKA has been solved [34]. The complete clone of AKAP-lbc was isolated in 2001 and was recognized to be a longer splice variant of the Lbc proto-oncogene that was most highly expressed in the heart [35]. The proto-oncogene of a transforming gene found in myeloid leukemias, Lbc is a Rho-specific guanine exchange factor (Rho-GEF). Following activation by G $_{\alpha 12}$ in lysophosphatidic acid-stimulated cells, AKAP-lbc stimulates Rho activity, resulting in stress-fiber formation in NIH3T3 cells.

AKAP-lbc activation of RhoA is inhibited by the binding of the small protein 14-3-3 when AKAP-lbc is phosphorylated by PKA at residue 1565 [36,37]. As a result, cAMP elevation will oppose the activation of AKAP-lbc-bound RhoA (Figure 2). α -adrenergic receptors activate both G $_{\alpha q}$ and G $_{\alpha 12/13}$ -dependent pathways in cardiac myocytes, each contributing to the induction of cellular hypertrophy [38]. AKAP-lbc is required for α -adrenergic-stimulated G $_{\alpha 12}$ activation of RhoA in myocytes [32]. Moreover, AKAP-lbc expression is required for α -adrenergic-induced cellular hypertrophy, ANF and α -skeletal actin expression.

AKAP-lbc vs. mAKAP β

AKAP-lbc and mAKAP β provide two important examples of signalosome scaffolds in cardiac myocytes that participate in distinct signaling pathways. Both scaffolds are required for induction of myocyte hypertrophy in vitro, and both bind PKA that is stimulated by β -agonists [30,39]. However, the complexes are quite distinct. While mAKAP β is associated with the nuclear envelope, AKAP-lbc has a more diffuse localization and is presumably near the plasma membrane. While mAKAP β is involved in calcineurin-NFATc signaling, AKAP-lbc is involved in RhoA signaling. Most strikingly, while PKA binding to mAKAP β is required for full induction of myocyte hypertrophy [30], one would predict that PKA bound to AKAP-lbc

would oppose myocyte hypertrophy [36]. Thus, while both scaffolds are sensitive to local cAMP, the functional consequences of elevated cAMP can be quite different in distinct areas of the cell. Bearing these facts in mind, one might conceive of custom therapeutic strategies that directly target either signalosome with different physiologic results.

Methods to Therapeutically Modify Signalosomes

Conventional drug therapy usually involves the application of an agonist or antagonist for a particular enzyme or receptor. The disadvantage of this approach is that the enzyme and receptor will be inhibited throughout the cell without regards to the function of individual pools, resulting potentially in untoward side effects. Strategies to specifically target individual signalosomes will need to be different if these strategies are to avoid the same side effects. Several approaches can be considered.

To begin with, the function of each signalosome is fundamentally dependent on the presence of the underlying scaffold. RNA interference (RNAi) is a method by which individual mRNAs are depleted with the cell, resulting in the depletion of the encoded protein [40]. RNAi is mediated by the introduction of small double stranded RNA oligonucleotides (siRNA) of which one strand is complementary to the target mRNA. siRNA will bind endogenous argonaute proteins, forming a RNA-induced silencing complex that will mediate the degradation of the desired target mRNA. In principal, any protein can be specifically targeted by RNAi. RNAi has been used in vitro to remove mAKAP β and AKAP-lbc from cultured myocytes in order to show that adrenergic-induced hypertrophy depends on the presence of these scaffolds [30,32, 41]. It may be possible to reduce pathologic remodeling in chronic heart disease by reduced expression of either of these scaffolds in vivo. It will be interesting to determine whether knock-out mice for these proteins are resistant to pathologic hypertrophy.

Human clinical trials are already underway for the use of RNAi in the treatment of macular degeneration and respiratory syncytial virus [40]. Current strategies for the delivery of siRNA include infection with defective viruses or the delivery of mature siRNA coupled to different types of carriers. A thorough review of the subject is beyond the scope of this discussion, but the reader is referred to de Fougères, et al. for a detailed discussion of the issues relating to in vivo RNAi [40]. Hurdles that will have to be overcome include the stability of siRNA in vivo, the ability to deliver the siRNA to the target tissue, the avoidance of the activation of innate immune response by siRNA and off-site miRNA-like effects.

If the scaffold cannot be depleted in vivo, it may be possible to remove the scaffold from its normal location, inhibiting its function. We have found that mAKAP β is targeted to the nuclear envelope through binding to nesprin-1 α . Overexpression of a polypeptide containing the spectrin repeat nesprin-1 α binding domain of mAKAP β will displace mAKAP β from the nuclear envelope [17]. Because mAKAP β is the only (known) AKAP to be localized through a spectrin repeat, it can be specifically displaced. Delocalization of mAKAP β resulted in the complete inhibition of cytokine-induced myocyte hypertrophy in vitro [22]. It may be possible to design a small molecule that specifically inhibits signalosome targeting.

A third method by which signalosomes may be targeted is the development of small molecules that may inhibit the binding of individual signaling components to the scaffold. This approach has been attempted with some success with regards to other protein binding events. For example, calcineurin is known to dephosphorylate many substrates. However, a subset of calcineurin substrates, including the NFATc transcription factor family, bind to a site distinct from the catalytic site of the phosphatase, such that calcineurin-NFATc binding is a prerequisite for enzyme action [42]. A peptide inhibitor, "VIVIT," has been derived that competes with high affinity for calcineurin binding to this set of substrates [43]. Although the VIVIT peptide

does not itself appear to be clinically useful, derivative peptides are being sought for that may allow the in vivo selective inhibition of calcineurin-dependent pathways. mAKAP β also binds calcineurin. Interestingly, the mAKAP β binding site for calcineurin is not similar to any other known binding site (M.S.K., unpublished observations). If so, it may be possible to develop another peptide that would specifically dissociate calcineurin A β from this scaffold, disrupting signalosome function and potentially inhibiting hypertrophy.

Conclusions

Cellular signaling is transduced by a complicated network of second messengers, protein kinases and other enzymes and ion channels. Current investigations directed towards the discovery of potential therapeutic mechanisms emphasize the identification of an enzyme or ion channel that may be the target of rational drug design and that may have a high therapeutic index. We would suggest that instead of targeting potentially pleiotropic enzymes it may be preferable to target the individual signalosomes that compose the different signaling pathways. By disruption of a signalosome, we may be able to selectively inhibit the germane functions of a particular enzyme or ion channel. While the application of RNAi to disrupt a signalosome only requires knowledge of the scaffold's mRNA sequence, the latter two methods discussed require a detailed understanding of the protein structure and function of signalosomes and their underlying scaffolds. These types of studies with regards to scaffolds such as mAKAP β and AKAP-lbc may yield novel strategies for the treatment of pathologic hypertrophy that will not inhibit myocyte contractility. Thus, understanding the complexity within the hypertrophic signaling network will aid in the development of more selective multi-targeted therapies that combine superior efficacy with improved safety.

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Figure 1. The mAKAPβ signalosome

mAKAPβ is localized to the nuclear envelope through binding to nesprin-1α. β-adrenergic receptor stimulation results in the production of cAMP through G_s-coupled adenylyl cyclase activation. cAMP-activated PKA phosphorylates and activates PDE4D3, attenuating PKA activation. α₁-adrenergic and gp130/leukemia inhibitory factor receptor stimulation results in MEK5 and ERK5 activation. ERK5 will phosphorylate and inhibit PDE4D3, synergistically activating PKA with cAMP. High levels of cAMP will oppose ERK5 activation via Epac1 and Rap1. PKA potentiates Ca²⁺-induced RyR2 Ca²⁺ release. Local Ca²⁺ may activate associated calcineurin Aβ (CaNAβ), which will dephosphorylate NFATc transcription factors, resulting in enhanced NFATc nuclear translocation and hypertrophic gene transcription.

Figure 2. The AKAP-lbc signalosome

AKAP-lbc is diffusely localized near the plasma membrane. AKAP-lbc activates RhoA in response to lysophosphatidic acid-stimulated $G_{\alpha 12}$. Activation of RhoA is inhibited by the binding of 14-3-3 following PKA phosphorylation of AKAP-lbc. Thus, cAMP elevation will oppose the activation of AKAP-lbc-bound RhoA.