Journal of the American Heart Association

CONTEMPORARY REVIEW

AKAP12 Signaling Complex: Impacts of Compartmentalizing cAMP-Dependent Signaling Pathways in the Heart and Various Signaling Systems

Hanan Qasim, BPharm; Bradley K. McConnell, PhD

ABSTRACT: Heart failure is a complex clinical syndrome, represented as an impairment in ventricular filling and myocardial blood ejection. As such, heart failure is one of the leading causes of death in the United States. With a mortality rate of 1 per 8 individuals and a prevalence of 6.2 million Americans, it has been projected that heart failure prevalence will increase by 46% by 2030. Cardiac remodeling (a general determinant of heart failure) is regulated by an extensive network of intertwined intracellular signaling pathways. The ability of signalosomes (molecular signaling complexes) to compartmentalize several cellular pathways has been recently established. These signalosome signaling complexes provide an additional level of specificity to general signaling pathways by regulating the association of upstream signals with downstream effector molecules. In cardiac myocytes, the AKAP12 (A-kinase anchoring protein 12) scaffolds a large signalosome that orchestrates spatiotemporal signaling through stabilizing pools of phosphatases and kinases. Predominantly upon β-AR (β_2-adrenergic-receptor) stimulation, the AKAP12 signalosome is recruited near the plasma membrane and binds tightly to β-AR. Thus, one major function of AKAP12 is compartmentalizing PKA (protein kinase A) signaling near the plasma membrane. In addition, it is involved in regulating desensitization, downregulation, and recycling of β-AR. In this review, the critical roles of AKAP12 as a scaffold protein in mediating signaling downstream GPCRs (G protein–coupled receptor) are discussed with an emphasis on its reported and potential roles in cardiovascular disease initiation and progression.

Key Words: adrenergic receptor ■ AKAP12 ■ compartmentalization ■ gravin ■ PKA ■ signaling pathways ■ signalosome

AKAPs (A-kinase anchoring proteins) (AKAPs) belong to a family of scaffolding proteins that organize complex signal transduction events descending from cell surface–stimulated receptors.[1] AKAPs mediate sequestering of protein kinases, phosphatases, and signal termination molecules, with their target substrates.[1] Thus, AKAPs coordinate phosphorylation and dephosphorylation states within the cell to confer the specificity of intracellular events. When first identified, regardless of the general structural diversity,[2] they were functionally characterized on the basis of their ability to bind to PKA (protein kinase A).[3] This binding capacity is mainly attributed to their highly conserved amphipathic helix that anchors to PKA regulatory subunits.[4] In addition, AKAPs localize to diverse subcellular locations, including, but not limited to, the plasma membrane, mitochondria, nuclear envelope, and cytoskeleton.[5–7]

Briefly, on their binding to the regulatory subunit of PKA, AKAPs localize the activation of PKA catalytic subunits, utilizing the specific targeting sequences located near their N terminus.[8] This explains how such a common signaling pathway (PKA signaling) can serve many different functions with high selectivity.

Correspondence to: Bradley K. McConnell, PhD, Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, 4849 Calhoun Road, Health-2 (H2) Building, Room 5024, Houston, TX 77204-5037. E-mail: bkmcconn@central.uh.edu

For Sources of Funding and Disclosures, see page 11.

JAHA is available at: www.ahajournals.org/journal/jaha

Nonstandard Abbreviations and Acronyms

AKAP	A-kinase-anchoring protein
β-AR	β-adrenergic receptor
ERK	extracellular signal–regulated kinase
GPCR	G protein–coupled receptor
PKA	protein kinase A
PKC	protein kinase C
PLN	phospholamban
SERCA	sarco/endoplasmic reticulum Ca^{2+}-ATPase
SR	sarcoplasmic reticulum
VIP	vasoactive intestinal peptide

However, later studies have shown that these scaffold proteins further can bind to other substrates and even homo- or heterodimerize/oligomerize,[9] indicating a more complex spatial and temporal signaling profile.[10–12]

Kinases and phosphatases are key regulators of enzymatic activities and protein interactions. AKAPs colocalize these enzymes by playing a major role in influencing cellular activities, where any deviation from their normal function may induce states of imbalance and disease. Several AKAPs have been identified within the past decade. However, their full function/mechanism of action in different systems under physiologic conditions and possible involvement in pathophysiologic states is not fully understood. Thus, in this review we covers some of the major AKAPs, with a stronger emphasis on AKAP12 and its possible associated cardiovascular pathways/effects.

AKAP–PKA COMMUNICATION PLATFORM

Recent studies have shown that distinct AKAPs bind to different protein kinases/phosphatases, uniquely colocalizing them to their targets. An exception is PKA, which is the common protein kinase that all AKAPs share the capacity to bind.[3] Considering the diverse network through which PKA signals, it is peculiar how it can still be highly specific to its targets. Further understanding of binding/signaling mechanisms through PKA gives insights into the general AKAP signalosome.

Briefly, PKA holoenzyme (a heterotetramer) contains 2 possible regulatory subunits, either RI or RII.[13] The regulatory subunits keep the enzyme in a dormant state at low levels of cAMP through inhibiting the 2 catalytic subunits (PKAc).[13] When PKAc subunits are activated, they phosphorylate serine/threonine residues on their multiple target substrates, triggering downstream signaling pathways.[14]

To manage a more distinct signal specificity of such diverse pathways, adjustments take place within the cell. Such adjustments include sequestration of AKAP complexes near their targets, a local increase in cAMP levels, and the appearance of termination mechanisms.[13,15] Although the local increase of cAMP levels postactivation of the upstream regulators of PKA may assure some level of specificity, yet another signal termination mechanism must exist to maintain homeostasis. AKAPs assure this through not only linking PKA to its upstream modulators but also to signal termination molecules such as phosphodiesterase through scaffolding to them as well.[16]

So far, all studied AKAPs contain the highly conserved sequence of 14 to 18 amino acids, usually Gln4–Lys21, which forms a 5-turn amphipathic (alpha) helix.[17] This helix is important because of its ability to slide into the binding pocket near the N terminus of PKA.[17,18] The majority of AKAPs preferentially anchor the RII dimer of the PKA holoenzyme.[19] However, D-AKAP1 and D-AKAP2 are capable of binding to either subtype of PKA-regulatory subunits.[19] Furthermore, recent studies have indicated that AKAP11 and sphingosine kinase–interacting protein selectively bind to the RIα subunit of PKA, forming a dynamic cytoplasmic signaling complex.[14,20] However, it remains unknown whether AKAP anchoring to either RI or RII subunits of PKA has differential effects on its signaling outcomes. Nonetheless, it is established that the binding of AKAPs to the RI or RII subunits neither affected the tertiary nor the quaternary conformations of the dimer.[18] On the contrary, exposure to elevated levels of cAMP induces such change.[21] Thus, AKAPs are not essential for the phosphorylation/activation of PKA. Rather they are crucial to spatially restrict the activation of PKA to areas where cAMP levels are high with the stimulation of upstream proteins/receptors.[21]

As mentioned previously, AKAPs can directly bind and sequester various signaling proteins. AKAPs can bind protein kinases (PKC [protein kinase C] and mitogen-activated protein kinase), phosphatases, cAMP phosphodiesterase (PDE), guanosine triphosphate–binding proteins, and adaptor proteins. PDEs reduce the local levels of cAMP available for triggering downstream signaling pathways.[22] Considering that PKA is mainly affected by cAMP levels, crosstalk between PKA and PDE is crucial to forming a feedback loop, which is believed to be mainly controlled through their colocalization by AKAPs at the same subcellular compartments.[23]

Table 1. AKAP-Reported Functions in Various Signaling Systems

System	AKAP	Effect	Mechanism
Nervous[32–34]	AKAP5 (AKAP79/150)	Promotes synaptic plasticity; essential for NMDA receptor–mediated LTD and normal motor coordination	Scaffolding of PKA, PKC, and PP2B to phosphorylate glutamate receptors
Immune[35,36]	Ezrin	Suppression of T-cell replication	Scaffolding of PKA to phosphorylate C-terminal Src kinase
	D-AKAP1	HIV progression	Cofactor of HIV reverse transcriptase
Reproductive[37–39]	AKAP1	Defective maturation of ovaries	Disrupted association of PKA to AKAP1
	AKAP3	Impaired sperm motility	PI3 interference with AKAP3–PKA binding
	AKAP4	Impaired sperm motility	Failure of association of glycolytic enzymes to the fibrous sheath
Endocrine[40,41]	AKAP79/150	Regulation of insulin secretion	Reduction of PP2B activity
	AKAP18α/γ	Enhance/reduction of insulin secretion respectively	Controlling glucagon-like peptide-1–mediated insulin secretion
Renal[42,43]	AKAP18δ	Trafficking of aquaporins AQP2 toward plasma membrane	PKA Phosphorylation at serine (S)256, S261, S264, and S269 at AQP2
	AKAP-Lbc	Redistribution of AQP2 from intracellular vesicles to the periphery of medullary collecting duct principal (IMCD) cells	PKA phosphorylation of RhoA at S188 inducing its inhibition
Respiratory[44]	AKAP1	Worsening of hyperoxia-induced acute lung injury	Inhibition of lung NF-κB p65 activity
Gastrointestinal[45,46]	AKAP150	Regulation of pepsinogen secretion	Unclear
	Ezrin	Parietal cell activation	Scaffolding of ezrin with Stx3
Liver[47]	Ezrin	Gap junction modulation	PKA phosphorylation of connexin 43

AKAP indicates A-kinase anchoring protein; AQP2 aquaporins; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate receptor; PKA, protein kinase A; PKC, protein kinase C; PP2B, protein phosphatase 2B; Stx3, syntaxin3; NF-κB, nuclear factor-kappa light-chain enhancer of activated B cells; PI3; phosphoinositide 3; SRC, proto-oncogene tyrosine–protein kinase; Stx3, syntaxin 3; and RhoA, Ras homolog family member A.

Another key signaling transduction mechanism that ultimately activates nuclear transcription factors is regulated by the calcium–calmodulin-dependent serine–threonine protein phosphatase 2B/calcineurin. Certain AKAPs, such as AKAP79/150, anchor protein phosphatase 2B/calcineurin to facilitate the downstream signaling. Ultimately, this signalosome complex activates effector molecules within the nucleus, including the nuclear factor of activated T cells.[24] This diversity of AKAPs and their complexes indicates their ability to control a vast array of opposing signaling pathways within the cells to maintain homeostasis. Regardless, the exact signaling mechanism driving their ability to compartmentalize the complexes near their targets is still speculative because of the high structural diversity of AKAPs and their anchoring domains. It is worthy of mention that the presence of adenylyl cyclase subtypes reported in some AKAP (AKAP79, Yotiao, and mAKAP) complexes[25] demonstrates a possible constitutional adenylyl cyclase–AKAP binding that maintains these unique clusters near their targets at basal levels where cAMP will be increased once the upstream receptors are stimulated. However, this scheme cannot be generalized for all AKAPs. For instance, myristylation of N-terminal sequences, along with the presence of basic amino acid–rich regions (polybasic domains) within the N terminus on AKAP12 is sufficient to mediate this localization near the β_2-AR.[26,27]

Notably, the AKAP12 association with PDE4D3 and β_2-AR (β_2-adrenergic receptor) recruitment was enhanced with activation of PKA.[23,28] PDE4 is exceptionally important in cardiovascular signaling, which is delineated by its signaling under cardiac diseases. For instance, under cardiac hypertrophy, PDE4 activity is decreased[29] and associated with propagation of heart failure.[30] Furthermore, acute PDE inhibition enhances cardiomyocyte function, whereas chronic inhibition results in increased mortality.[31] This complexity may be explained by the compartmentalized controls of cAMP levels by PDE4, in an agonist-dependent manner downstream of β_2-AR. Such properties make PDE4 a favorable partner for many AKAPs.

AKAP FUNCTIONAL ROLES

Generally, signaling interactions are governed by the global abundance of proteins in addition to their specific interactions. The specificity of such interactions is fundamental to maintain homeostasis and is controlled by the organized subcellular microdomains, coordinated by several scaffolding proteins, some of which are AKAPs. AKAPs dictate how, when, and where each member of their complexes will initiate a

certain action. Usually, under pathophysiologic conditions, the global balance of signaling molecules, such as cAMP and PKA, is not affected. Rather imbalances within certain AKAP complexes are evident, thus making them valuable therapeutic targets. In what follows we briefly cover the involvement of different AKAPs in variable systems (Table 1),[32–47] followed by a more detailed discussion of their functions in the cardiovascular system.

AKAPS AND CARDIOVASCULAR SYSTEM

Cardiac function is strongly impacted by the adrenergic system, mainly through the interaction of catecholamines, with β-ARs controlling both contractility and heart rate.[48] Such distinct cellular events are mainly controlled via PKA followed by the secondary messenger cAMP, on stimulation of GPCRs (G protein–coupled receptors).[49] Importantly, several reports showed that cAMP levels increase within specific cellular compartments, as a "local increase" in a stimulus-induced manner, assuring some level of specificity.[50–52] Also, multiple AKAPs have been associated with the compartmentalization of cardiac signaling pathways. Of the >50 AKAPs characterized so far, several have been associated with cardiac development, contractility, cardiac morphology, and rhythm (Table 2).[53–64]

Cardiac Development

During cardiac development (Figure 1),[62,65] AKAP-LBC (AKAP13) is postulated as a key regulator of cardiomyocyte differentiation and morphogenesis.[65] The mechanism of this regulation of differentiation and morphogenesis is believed to be through producing a platform that links Gα12 and Rho signaling to an essential transcriptional program, as confirmed by the observed embryonic lethality upon deletion of AKAP13 in mice.[66]

Cardiac Contractility

Regarding cardiac contractility (Figure 2), normal contraction of the heart is controlled by a delicate regulation of cytosolic Ca^{2+} levels through a complex interplay between the ryanodine receptor, L-type calcium channels, SR (sarcoplasmic reticulum), and SERCA (sarco/endoplasmic reticulum Ca^{2+}-adenosine triphosphatase). Several AKAPs have been involved in facilitating the proper signal transduction within these complexes, such as AKAP79/150, AKAP12, AKAP15/18, mAKAP, and AKAP18δ. For instance, Ca^{2+} signaling on the surface of the cell was linked to AKAP15/18 ability to target the PKA to the C terminus of L-type voltage-gated calcium channel channels. Nuclear Ca^{2+} signaling is regulated through mAKAP acting on the nuclear membrane, whereas AKAP18δ affects SR levels of Ca^{2+}.[66] mAKAP forms macromolecular complexes, composed of PKA, PDE4D3, adenylyl cyclase 5, protein phosphatase 2A, mitogen-activated protein kinase 5, ERK5 (extracellular signal–regulated kinase 5) Rap1, and Epac1.[67–70] The strategic location of this complex on the nuclear membrane allows mAKAP to promote the opening of ryanodine receptor channels located on the nuclear envelope (through targeting

Table 2. Cardiac AKAP-Reported Functions

AKAP	Alternative Names	Effect	Mechanism
AKAP1[53]	D-AKAP, AKAP121	Reduction of ROS in the heart	Increase SOD2 mitochondrial expression
AKAP5[53]	AKAP79, AKAP75, AKAP150	Increase ROS, and inflammatory responses	Activation of PKC
AKAP6[54]	AKAP100, mAKAP	Regulation of cardiomyocyte oxygen homeostasis	Enhances the transcriptional activation of HIF-1α–regulated genes
AKAP7[55]	AKAP15, AKAP18	Regulation of calcium handling	Coordinates PKA phosphorylation of PLN
AKAP9[56]	Yotiao	Increases the slow outward potassium ion current	Targeting PKA and PP1 to hKCNQ1
AKAP10[57]	D-AKAP2	Regulation of cardiac rhythm	Unknown
AKAP12[58,60,61]	Gravin, SSeCKS	Cardiac pathophysiology (*to be defined*)	Unknown
AKAP13[53,62]	AKAP Lbc	Mediates metabolic switch during the development of compensatory hypertrophy	Activation of PKD1
Synemin[63]	SYNM	Maintenance of normal ventricular function	Unknown
Myosprin[64]	CMYA5	Modulates the clustering of RyR	Unknown

AKAP indicates A-kinase anchoring protein, HIF-1α, hypoxia-inducible factor-1α; hKCNQ1, human potassium voltage-gated channel subfamily Q member 1; PKA, protein kinase A; PKC, protein kinase C; PKD1, polycystin 1; PLN, phospholamban; PP1, protein phosphatase 1; ROS, reactive oxygen species; RyR, ryanodine receptor; and SOD2, superoxide dismutase 2.

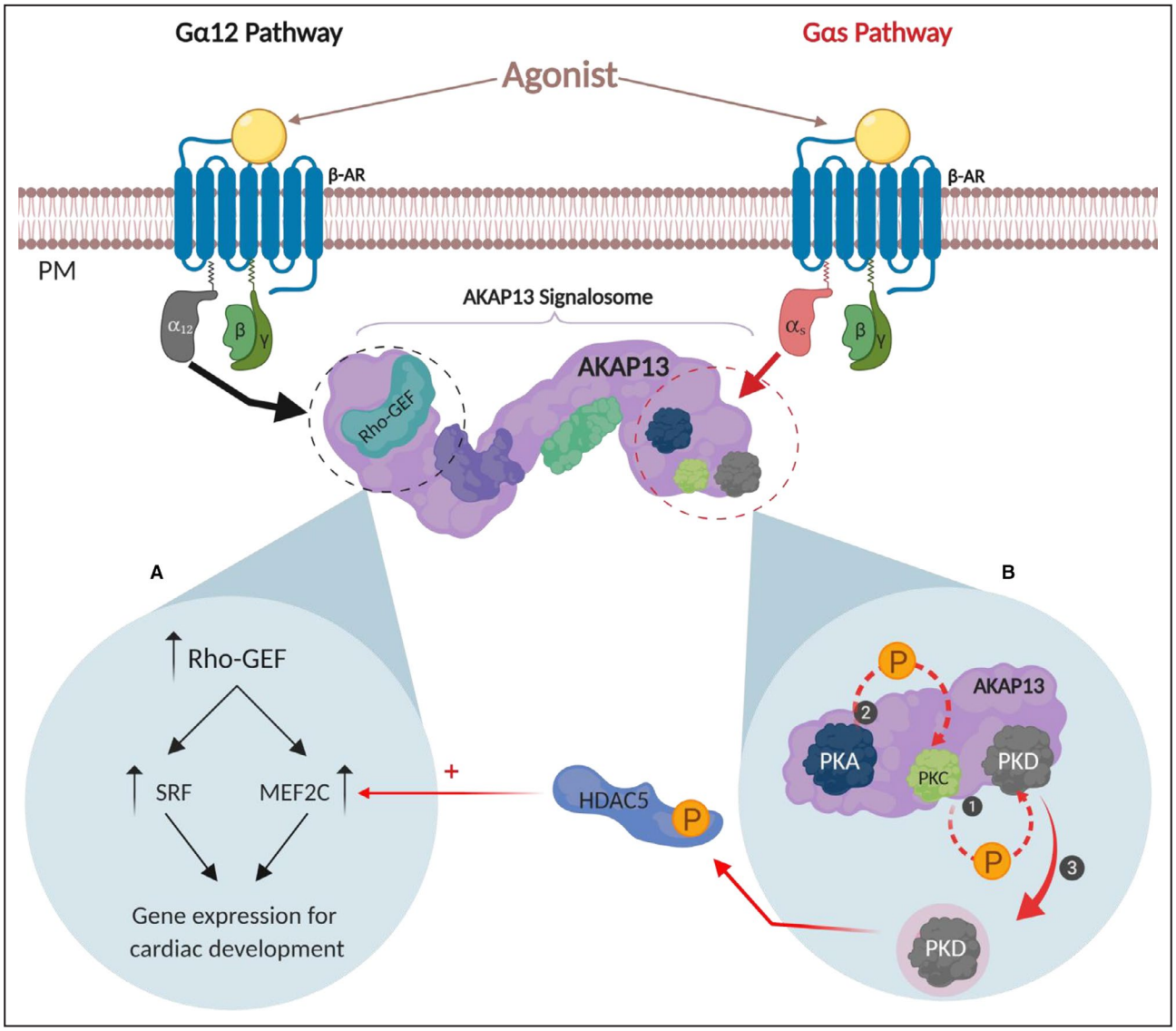

Figure 1. Role of AKAP13 in cardiac function.
GPCR signaling is directed by the type of ligand binding to this receptor. The nature of the ligand would favorably shift the equilibrium to a specific conformation of the receptor, which signals through a distinct G protein. For AKAP13 and GPCR signaling in the heart, it has been associated with 2 distinct G protein–signaling pathways (Gα12 and Gαs). **A**, When stimulation of β-AR stabilizes its conformation to signal through Gα12, AKAP13 signalosome is attracted near the receptor. AKAP13 interacts with Gα12 allowing Rho-GEF stimulation. Upon Rho-GEF stimulation, both SRF (Rho-GEF guanosine triphosphatase) and myocyte enhancer factor 2C (transcription factor) activities are increased. However, the mechanism of their activation depends on different regions of AKAP13. Activation of myocyte enhancer factor 2C depends on the carboxyl region of AKAP13 (BRX), whereas SRF activation depends on the GEF region of AKAP13. Collectively, this suggests that increased gene expression of factors associated with cardiac development may be partially dependent on AKAP13.[65] **B**, AKAP13 has been reported in the cardiac hypertrophic signaling. Under states of increased hemodynamic load, it is usually accompanied by a favorable state of Gαs pathway signaling within the cardiomyocytes. AKAP13 signalosome releases PKD to phosphorylate HDAC5. Phosphorylation of HDAC5 activates MEF2, which drives the transcription of genes associated with cell growth, Ca^{2+} handling, and contraction. The aforementioned multistep process of PKD release starts with PKA activation as a response to the Gαs pathway. PKA further phosphorylates PKC. Phosphorylation of PKC activates PKD, leading to its dissociation from the signalosome.[62] α12 indicates α subunit of Gα12 protein; αs, α subunit of Gαs protein; β-AR, β-adrenergic receptor; AKAP, A-kinase anchoring protein; GPCR, G protein–coupled receptor; HDAC5, histone deacetylase 5; MEF2C, myocyte enhancer factor 2C; PKA, protein kinase A; PKC, protein kinase C; PKD, protein kinase D; PM, plasma membrane; Rho-GEF, Rho guanine nucleotide exchange factor; and SRF, serum response factor.

PKA to these channels using leucine/isoleucine zipper motifs within the mAKAP), impacting both Ca^{2+} rise and perinuclear flux to further increase cardiac function.[66,71] Historically, mAKAP was thought to play a crucial role in cardiac contractility on the basis of studies identifying its localization on the SR.[72,73] However, later studies alternatively have established that mAKAP is located on the nuclear membrane

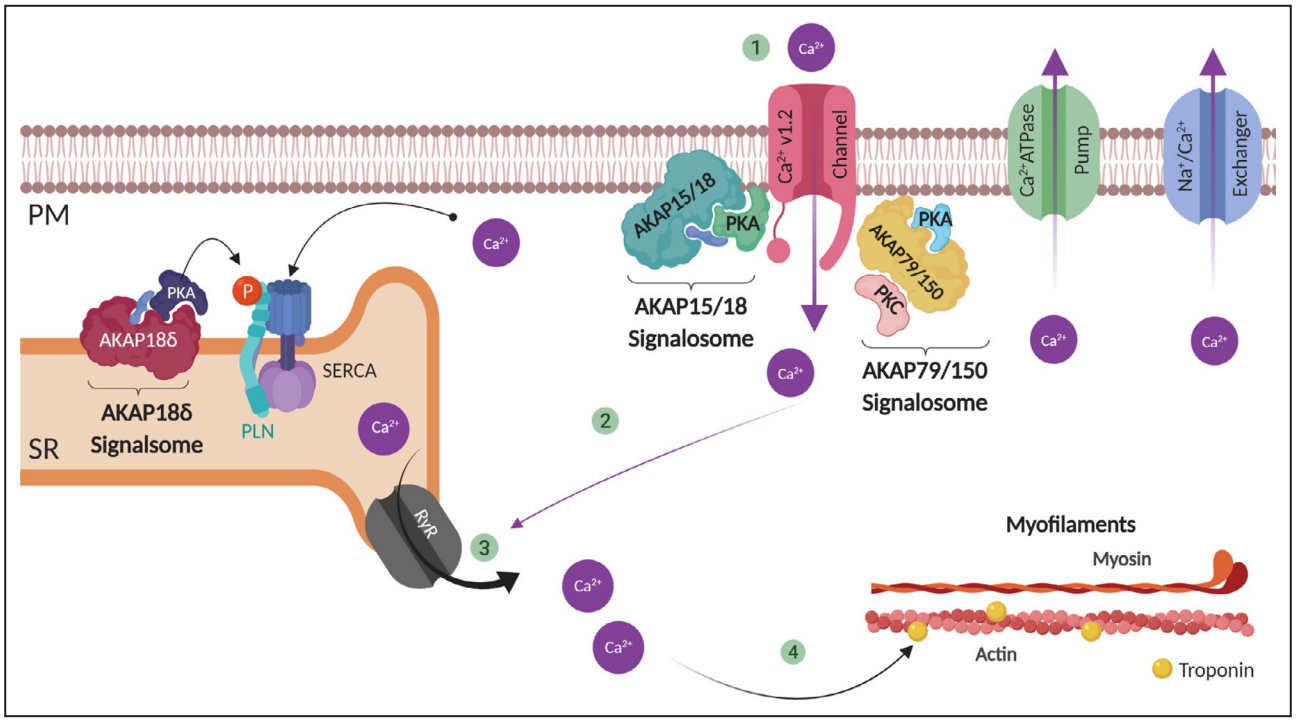

Figure 2. Role of AKAP complexes in cardiac contractility.
Cardiac contractility is controlled mainly by the delicate interplay between RyR, calcium channels ($Ca^{2+}_v1.2$), SR, and SERCA. On electrical stimulation, the voltage-gated Ca^{2+} channels increase Ca^{2+} influx, leading to local areas of high Ca^{2+} (**1**). The local increase of Ca^{2+} activates the RyR channels located on the SR (**2**). The RyR channels activation increases Ca^{2+} efflux from the SR, causing a cytosolic (global) increased Ca^{2+} (**3**). The global increase in Ca^{2+} triggers muscle contraction cycle, as a result of Ca^{2+} binding to troponin (**4**). SERCA plays a major role in maintaining consistency in the contractile force between cardiac beats. As a response to high cytosolic levels of Ca^{2+}, SERCA reuptakes Ca^{2+} back into the SR to return to baseline levels in the cytoplasm. SERCA activity is controlled by PLN, which, when phosphorylated by PKA, allows SERCA to reuptake Ca^{2+}. AKAPs play a crucial role in each of these phases; AKAP15/18 facilitates Ca^{2+} influx through targeting PKA to the C terminus of $Ca^{2+}_v1.2$ channels, AKAP79/150 binds PKA and PKCα to $Ca_v1.2$ channels to facilitate their normal gating, and AKAP18δ mediates PKA phosphorylation of PLN causing it to detach from SERCA, thus allowing the reuptake of Ca^{2+}. AKAP indicates A-kinase anchoring protein; $Ca^{2+}_v1.2$, calcium channel; Na^+/Ca^{2+} exchanger, sodium–calcium exchanger; PKA, protein kinase A; PKC, protein kinase C; PLN, phospholamban; PM, plasma membrane; RyR, ryanodine receptor; SERCA, sarco/endoplasmic reticulum Ca^{2+}-adenosine triphosphatase; and SR, sarcoplasmic reticulum.

rather than SR and it acts on the nuclear ryanodine receptor.[74,75] Considering the differences in the signaling between cytoplasmic and nuclear Ca^{2+} pools, mAKAP is primarily involved in gene expression regulation rather than cardiac contractility.[76]

AKAP18δ influences SERCA2 needed for reuptake of Ca^{2+} from the cytoplasm to assure consistency of cardiac contractile force within each beat. Briefly, SERCA2 activity is affected by calcium gradients, level of SERCA2 expression, and the levels of the SERCA2 suppressor PLN (phospholamban). In this manner, AKAP18δ mediates PKA phosphorylation of serine 16 on PLN, dissociating it from SERCA2, which subsequently increases the uptake of Ca^{2+} into the SR and enhances cardiac function.[55] Other AKAPs, such as synemin[77] and myomegalin, have also been proposed to play a role in cardiac contractility because of their sarcomere intracellular localization.[77,78]

In addition to the aforementioned function of AKAP79/150 in cardiac contractility, it has been also linked to cardiac rhythm regulation.[79] This regulation of cardiac rhythm is potentially achieved through binding PKA and PKCα to L-type voltage-gated calcium channels to facilitate their normal gating, thus protecting against arrhythmias.[79] Moreover, Yotiao (AKAP9) regulates normal cardiac rhythm by recruiting PKA and protein phosphatase-1 to the C-terminal subunit of the slowly activating delayed rectifier potassium channels, where these channels are responsible for conducting ionic currents.[80] Finally, D-AKAP2 (AKAP10) was proposed to control pacemaker sensitivity to cholinergic stimulation.[57] It is has been proposed that a coordinated interplay between these 3 AKAPs (AKAP79/150, Yotiao, and D-AKAP2) regulates heart rhythm in distinct yet interconnected pathways.

Cardiac Hypertrophy

Cardiac hypertrophy, defined as an increase in cardiomyocyte size (adaptive remodeling of the heart) under stress or continuous stimulation, can develop into further pathophysiologic states, such

as heart failure. Thus, the involvement of mAKAP, AKAP79/150, and AKAP-LBC was examined in mediating the pathologic state of cardiac hypertrophy. So far, mAKAP and AKAP79/150 activation has been associated with nuclear translocation of nuclear factor of activated cells, promoting the transcription of hypertrophic genes.[81] In addition, mAKAP activates the mitogen-activated protein kinase pathway in a cAMP-dependent manner, which entails further activation of the prohypertrophic factor myocyte enhancer factor 2C.[69] Alternatively, AKAP-LBC (AKAP13) facilitates PKC/PKD coupling to inactivate histone deacetylase to induce cardiac hypertrophy (Figure 1).[82]

Together, it is evident from the accumulated studies that AKAPs play a major role in controlling many aspects of cardiac function, and that any alteration within their complexes may contribute to the development of cardiac disease. This notion is supported by the important roles reported on other AKAP signalosomes in developing cardiac hypertrophy. For instance, in heart failure there is upregulated gene expression of AKAP-LBC, AKAP18, and AKAP2, whereas the AKAP121 gene was downregulated.[83] Interestingly, AKAP12 (detailed in what follows) knockout ameliorated cardiac function in an animal heart failure model[58]; however, the exact mechanism is not yet fully understood, and more research is needed to elucidate this observation.

Importantly, AKAPs may be involved in alterations of spatial localization of β-AR in heart failure. In failing hearts, β_2-AR signaling is redistributed from T tubules to cell crest. As a result, β_2-AR uncouples localized PKA pools, mediating a cellwide rather than localized cAMP propagation.[84] Such alteration in signaling further propagates the heart failure phenotype. As of now, roles of AKAPs in mediating such responses in cardiomyocytes are remain unknown.[85]

β_2-AR dysregulation is associated with heart failure.[86,87] Stimulated β_2-ARs couples with Gαs and can subsequently switch to Gαi, regulating cAMP levels, PKA activity, and phosphorylation of several downstream pathways.[88] Alterations in β_2-AR desensitization and resensitization play a major role in development of heart failure. Mechanisms of β_2-AR desensitization are well characterized,[89-92] but few studies have investigated β_2-AR resensitization schemes.[92–94] Notably, one study reported the importance of Ser-355/356 phosphorylation in regulating physiologic resensitization in neonatal cardiomyocytes.[93] Another study using an integrated whole-cell response microfluidic system suggested the need for Src-regulated processes and dynamin (cytoskeleton-associated protein) for β_2-AR de/resensitization.[92] Interestingly, elevated AKAP121 levels reduced apoptosis of cardiomyocytes understimulated ischemia, through its action on dynamin-like receptor-1.[95] Accordingly, reports of failing human hearts showed diminished levels of AKAP121.[83,96]

Collectively, AKAP complexes contain multiple proteins that play a significant role in regulating β-AR signaling and can be a valuable therapeutic target, as they contribute to mediating several cardiac functions. Thus, future studies should focus on the biochemical details of these protein networks and their involvement in both physiologic and pathophysiologic aspects.

AKAP12 IS AN A-KINASE ANCHORING PROTEIN

AKAP12 was first recognized as an autoantigen in serum from myasthenia gravis patients,[97] hence the alias name (Gravin). It is also known as the Src-suppressed C kinase substrate, which is considered the rodent ortholog of the human identified Gravin[98]; however, these terms are used interchangeably. This 250-kd multivalent AKAP interacts with PKA, along with several other molecules and signaling proteins such as PKC, β_2-AR, PDE4D, β-1,4-galactosyltransferase, non-receptor tyrosine kinase Src, and Ca^{2+}/calmodulin. The AKAP12 signalosome plays a central role in organizing (GPCRs) to protein kinases and phosphatases. In particular, it targets PKA and other signaling molecules to the cell periphery near β_2-ARs, to regulate its resensitization and recycling.[99–101]

It has been proposed that AKAP12 subcellular distribution is dynamic and affected by intracellular Ca^{2+} concentration, in addition to the level of PKC activation. This introduces the possibility of crosstalk between PKA, PKC, Ca^{2+}/calmodulin,[99,100] and potentially other additional molecules.

Upon evaluating AKAP12's structure (Figure 3), [102–104] domains responsible for its targeting to the cell periphery are believed to be the N terminal's myristoylation sites along with its 3 polybasic domains located near the N terminus.[26] However, the exact mechanism is still unclear. In particular, one study reported that the presence of the polybasic subunits was sufficient to only dissociate AKAP12 from the cell periphery in response to phorbol ester treatment, whereas the putative N-terminal myristoylation was required for AKAP12's redistribution to intracellular vesicular compartments.[26] Yet another study demonstrated that the presence of any 2 polybasic domains, as described in their constructs lacking the myristoylation sites, was sufficient for targeting AKAP12 to the cell periphery and subsequent regulation of β_2-AR resensitization.[100]

Furthermore, 3 distinct isoforms of AKAP12 (α, β, or γ) have been identified so far and they share

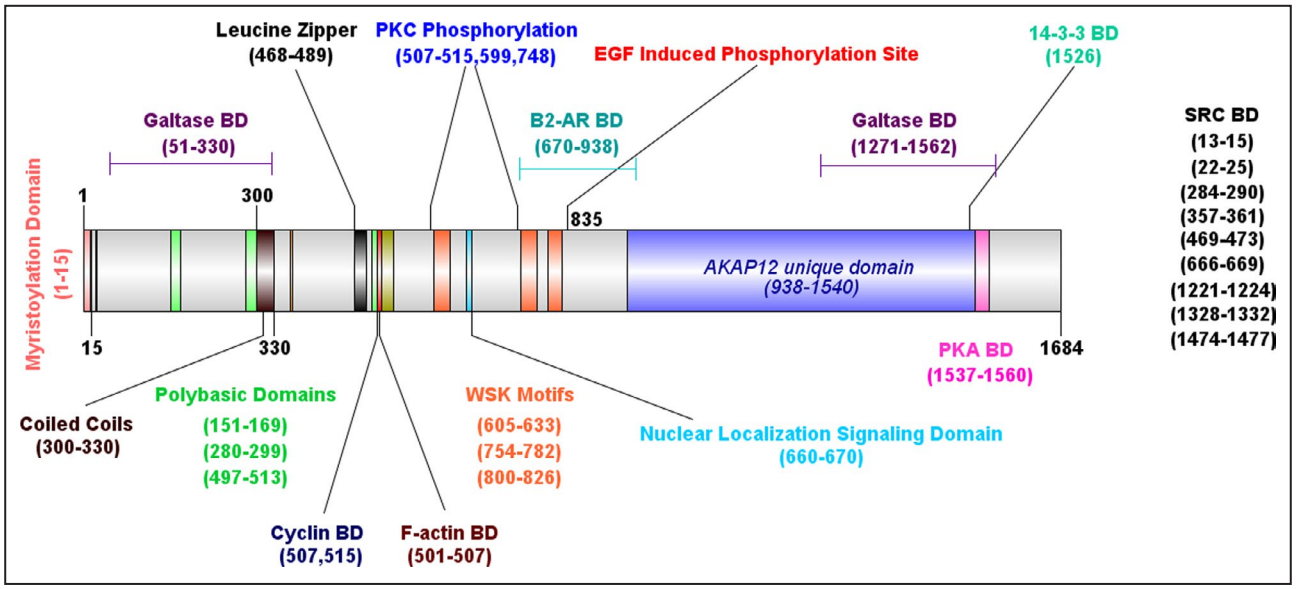

Figure 3. AKAP12 schematic topological map shows the known and potential protein–protein binding and phosphorylation domains.

2–7 coiled coils indicates 2–7 alpha-helices usually involved in gene expression[102-104]; β2-AR indicates β_2-adrenergic receptor; BD, binding domain; EGF, epidermal growth factor; GalTase, β-1,4-galactosyltransferase; PKA, protein kinase A; PKC, protein kinase C; SRC, non-receptor tyrosine kinase; and WSK, short motifs composed of 3 conserved residues found in a WXSXK motif.

>95% amino acid sequence identity, with variability between the isoforms only shown to be within their N terminus. Thus, having 3 AKAP12 isoforms can potentially explain the distinct spatial distribution isoform profiles such that the myristoylation of an AKAP12α is necessary for targeting it to the endoplasmic reticulum.[105] Notably, these isoforms are independent transcripts under the control of separate promoters, showing distinct spatiotemporal mRNA expression in different organs.[105]

In summary, these 2 features of AKAP12---(1) the presence of many docking/binding domains for kinases/phosphatases and (2) its broad expression---drives its ability to influence cellular signaling pathways in many systems. Thus, these cellular signaling roles of AKAP12 are discussed in more detail, along with an emphasis on its cardiac capacities, in what follows.

AKAP12 SIGNALING IN CELLULAR SYSTEMS

AKAP12 is probably one of the most studied AKAPs in cell cycle regulation. It is known as a negative regulator of the G1/S-phase transition and important for the completion of cytokinesis through altering expression levels of cyclin D1 by either blocking its synthesis[106] or scaffolding PKC to control the formation of actin–myosin rings.[107] Accordingly, AKAP12 was intensively studied in the cancer field and reported as a metastasis suppressor in a variety of cancers,[102] such as prostate and skin cancers.[108] Metastasis suppression has been attributed to attenuating tumor-intrinsic PKC and Src pathways[108] or reducing the secretion of tumor chemoattractants in the peritoneum.[106] In contrast, a study examining the mechanism underlying glioblastoma multiform reported that patients with higher AKAP12 mRNA levels had overall worse survival rates.[109] Although AKAP12 expression eas shown to be downregulated in several cancers, including prostate, breast, gastric, skin, and colon cancers, in addition to hepatocellular carcinoma and melanoma, its upregulation may still be a risk factor in other types of cancer.[109]

When examining the roles of AKAP12 in endothelial tissues, it has been proposed that vascular endothelial dysfunction, generated by lipopolysaccharide treatment, induces upregulation of AKAP12. Such upregulation of AKAP12 may improve endothelium-dependent relaxation through its binding to the atypical isoform of PKC (PKCζ), a well-known pathologic mediator of endothelial dysfunction.[110] Upon binding, AKAP12 inhibits PKCζ-mediated reduction of the ERK5 pathway.[110] Furthermore, depletion of AKAP12 was shown to increase pro-inflammatory proteins (tumor necrosis factor-α, interleukin-1β, and interleukin-6) and reactive oxygen species formation while reducing both endothelial nitric oxide synthase expression and ERK5 transcription.[110] This suggests the potential participation of AKAP12 in altering cytoskeletal morphology during inflammation.

In addition, lipopolysaccharide-induced inflammatory response in astrocytes has revealed that the β-1,4-galactosyltransferase-I–AKAP12 complex colocalizes in the perinuclear region at either basal levels or in the presence of lipopolysaccharide.[111] The complex was directed to the trans-Golgi network, where astrocyte migration was inhibited during lipopolysaccharide treatment.[111] This may be explained by inhibition of β-1,4-galactosylation of integrin-β1, suggesting that AKAP12 integrates the various functions attributed to the galactosyltransferase I cytoplasmic domain.[111]

In line with these findings, AKAP12 has been associated with macrophage regulation during the convalescent stage of inflammation. In particular, this was shown by AKAP12 activating the M2 macrophages (anti-inflammatory type), which then induced a more compact extracellular matrix. This was further confirmed by the positive correlation between both CD206 (M2 marker) and AKAP12 expression in humans and animal models.[112] Interestingly, a recent study investigating GPCR-mediated transactivation of epidermal growth factor receptor in epithelial cells showed that AKAP12 stabilized naked cuticle homolog 2 by facilitating PKA phosphorylation of Ser-223 on naked cuticle homolog 2. This further ensured efficient cell-surface delivery of transforming growth factor-α to increase epidermal growth factor receptor activation. Furthermore, AKAP12 knockout canceled baseline phosphorylation of the naked cuticle homolog 2 domain and reduced it in response to forskolin, VIP (vasoactive intestinal peptide), and prostaglandin estradiol,[113] stipulating a potential constitutive phosphorylating capacity of AKAP12 within its domains. Considering the homeostatic capacity of epithelial cells within multiple organs, further studies are crucial to examine such constitutive activity.

Within the pulmonary smooth muscles, the main signaling pathways are governed by coupling of the G protein, either Gαs or Gαq, to β-ARs. The Gαq pathway controls smooth muscle contraction and tone through activating phospholipase C and diacylglycerol, whereas the Gαs pathway causes local elevation of cAMP, which further causes activation of PKA and Epac in airway smooth muscles to influence contraction/relaxation of these muscles.[114] AKAP12 and AKAP5 are the main mediators of compartmentalizing phospholipase C, diacylglycerol, and PKA near the β_2-ARs and muscarinic receptor 3. AKAP5 increases G protein–coupled receptor kinase 2 recruitment to the cell surface, causing β_2-AR desensitization and internalization. AKAP12, however, is not involved in agonist-specific desensitization or the internalization of GPCRs.[115–117] Instead, it is responsible for the dephosphorylation, resensitization, and recycling of these receptors from intracellular vesicles back to the periphery. Thus, a balance between these AKAPs in addition to AKAP78 (involved in β_2-AR internalization) is needed for proper function of the airways.[118,119]

In addition, both AKAP12 and AKAP5 have been proposed to be regulators of pulmonary endothelial barrier function, and AKAP12 is required for this cAMP-mediated barrier stabilization.[120,121] However, a study examining the mechanism of cigarette smoke extract deterioration on airway endothelial function showed that mRNA expression levels of AKAP12 and AKAP5 were not affected, whereas a reduction in expression levels of another AKAP member (AKAP9) was noted and associated with reduced E-cadherin levels.[122] Interestingly, another study linking hypoxia in endothelial cells, widely implicated in many pathologic conditions, has also been linked to elevated AKAP12 gene expression, which is in contrast to other members of the AKAP family (AKAP1, AKAP17A, AKAP79, and AKAP100). Moreover, an analysis of the cloned promoter of AKAP12 revealed a functional hypoxia-responsive domain with 2 binding sites for hypoxia-inducible factor.[123]

Furthermore, AKAP12 gene expression has also been inversely associated with estrogen receptor gene expression, particularly Erβ.[124] In Erβ-null granulosa cells, upregulation of AKAP12 genes was identified as a mechanism that reduced cAMP levels through combating the sequestration of PKA regulatory subunits.[124] This was further supported in a study examining the agonist-dependent AKAP12 role in regulating cAMP levels. The study demonstrated that AKAP12 expression is crucial to allow (human embryonic kidney 293 cell line and human epidermoid carcinoma cell line) cells to recover their cAMP response to the agonist.[118]

Overall, it is apparent that AKAP12 plays a critical role in regulating multiple signaling pathways, but further studies are necessary to elucidate its role under different conditions.

AKAP12 ROLE IN CARDIOVASCULAR SYSTEM

In contrast to the extensive studies involving AKAP12's role in cancer research, there is little knowledge of its role in the cardiovascular system. AKAP12 is highly expressed in the heart[103] and functions to scaffold its signalosome complex near β_2-AR to influence resensitization and sequestration events.[116] Usually, scaffold proteins physically and transiently tether their complexes. However, AKAP12/β_2-AR interaction is unique as it was found to be strengthened upon stimulation of β_2-AR and remains intact even during the internalization of these receptors.[59] Considering that cardiac function

is mainly influenced by β-Ars, and that AKAP12 directly binds to β_2-AR, it is logical to assume that AKAP12 plays a role in regulating cardiac function.

To date, few studies have investigated AKAP12's involvement in cardiac pathophysiology, with the majority of studies indicating that AKAP12 downregulation improves cardiac function. For example, downregulation of AKAP12 reduced ventricular remodeling after chronic myocardial infarction (MI) surgery in transgenic mice overexpressing thioredoxin-1. This is believed to be associated with AKAP12's regulation of redox-sensitive transcription factor hypoxia-inducible factor-α.[125] Hypoxia-inducible factor-α is a critical expression regulator of both vascular endothelial growth factor and endothelial nitric oxide synthase, where AKAP12 destabilizes hypoxia-inducible factor-α by enhancing its proteosomal degredation.[126] Thus, the thioredoxin-1 cardioprotective effects of overexpression are partially attributed to reduced oxidative stress–mediated stabilization of hypoxia-inducible factor-α, which thereby increases vascular endothelial growth factor and endothelial nitric oxide levels.[126]

Knowing that cardiac hypertrophy and fibrosis are strongly associated with increased mortality in diabetic patients, the role of vascular endothelial growth factor, endothelial nitric oxide, and heat shock proteins in relation to AKAP12 were studied in the diabetic milieu of post-myocardial infarction (MI) surgery. Surprisingly, the study confirmed that heat shock protein A12B attenuated AKAP12 action, mediated cardioprotection through upregulating (thioredoxin-1), and suggested AKAP12

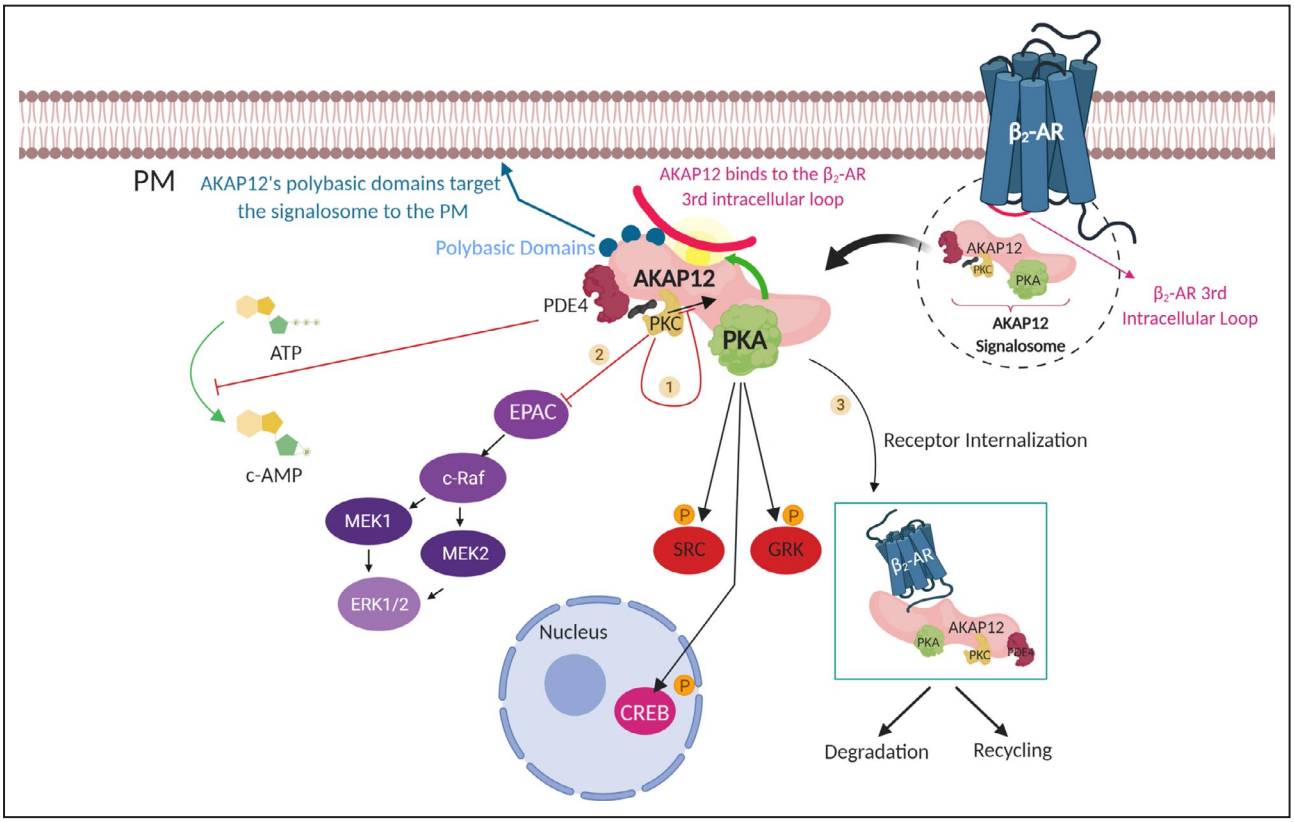

Figure 4. Proposed AKAP12 signaling downstream of β2-AR.
AKAP12 scaffolds multiple kinases, in addition to polybasic domains, which control its interactions and signaling. Under the unstimulated β_2-AR state, AKAP12 is found mainly within the cytoplasm. However, when β2-AR is stimulated, the polybasic domains found within the AKAP12 signalosome targets the complex to the cell membrane. This colocalization of the signalosome facilitates a stable binding of β_2-AR and AKAP12. Activation of PKA is associated with increased levels of cAMP in its proximity. β_2-AR stimulation induces local increases of cAMP. AKAP12 is important for PKA activation through scaffolding and collocating PKA near areas of high cAMP levels. The active form of PKA phosphorylates multiple downstream molecules, such as CREB (cellular transcription factor), SRC (tyrosine kinase), and GRK (G protein kinases), all playing a role in downstream signaling of β_2-AR. Importantly, PKA phosphorylates AKAP12, which stabilizes the interaction between AKAP12 and the β_2-AR, even during post-internalization of the receptor.[102] On the other hand, phosphorylation of AKAP12 by PKC decreases AKAP12–PKC scaffolding (**1**),[99] inhibits the PKC-Raf/MEK/ERK pathway (**2**),[129] and induces translocation of the AKAP12/PKA/β2-AR complex to the perinuclear space (**3**).[99] AKAP indicates A-kinase anchoring protein; β2-AR, β_2-adrenergic receptor; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CREB, cellular transcription factor; EPAC, exchange protein directly activated by cAMP; ERK, extracellular signal–regulated kinase; GRK, G protein kinase; MEK, mitogen-activated protein kinase; PDE4, phosphodiesterase-4; PKA, protein kinase A; PKC, protein kinase C; PM, plasma membrane; Raf, rapidly accelerated fibrosarcoma; and SRC, tyrosine kinase.

involvement in the downregulation of thioredoxin-interacting protein. In addition, on the basis of previous reports of heat shock protein A12B binding directly to a specific domain within AKAP12 (829–860 amino acid residues) located between the N and C terminus,[101] cardioprotection may be induced through genetic modifications causing AKAP12 downregulation. Taking into account that AKAP12 downregulation affected the cycling of the β_2-AR, this observation likely occurs through reduced recruitment of proteins involved in receptor desensitization (G protein–coupled receptor kinase 2 and β-arrestin).[28]

In efforts to better understand the mechanism of AKAP12 in β_2-AR signaling pathways, AKAP12-t/t mice were examined after acute infusion with the nonspecific β-AR agonist isoproterenol.[58,59] In contrast to a study fully ablated AKAP12,[60] AKAP12-t/t only lack the critical region required for β_2-AR, PKA, or PKC binding.[58,59] This reduces the probability of influence of the upregulated compensatory mechanisms, as in full-knockout AKAP12 gene models. Acute β-AR stimulation of AKAP12-t/t delineated enhanced contractility and basal activity. The proposed mechanism of increased basal activity was because of increased cMyBPC Ser-273 phosphorylation by heat shock protein 20, although increased cardiac contractility could be associated with altering PKA phosphorylating capacity as well as activity of PDE4D.[58] Also, it was suggested that AKAP12-t/t muscle exhibited increased myofilament responsiveness to Ca^{2+},[59] supporting the concept of AKAP12 being more than just a scaffold protein.

An additional study speculated that AKAP12 ablation aggravated heart failure induced post–angiotensin II infusion by promoting oxidative stress, apoptosis, cardiac fibrosis, and inflammatory response.[60] In that study, the authors suggested that the mechanism of increased cardiac fibrosis was mainly through increased levels of transforming growth factor-β1 and collagen, as well as through activation of the SMAD2/3 pathway.[60]

In addition to these findings, it has been reported recently that AKAP12-t/t mice exhibit a delayed high-fat-diet–induced hyperlipidemia and atherosclerosis.[61] It is believed that such resistance to hyperlipidemia and atherosclerosis development is associated with reduced gene expression of sterol regulatory element-binding protein 2. Further reducing gene expression of liver 3-hydroxy-3-methyl-glutaryl-CoA reductase and low-density lipoprotein receptor.[61] Consistent with these findings, Choi et al reported that overexpression of AKAP12 is involved in sterol regulatory element-binding protein 2 activation in a SCAP-dependent manner,[59,127] probably through enhancing [3H]-cholesterol efflux to extracellular acceptors. These findings further argue in favor of the AKAP12-t/t cardioprotective phenotype from a pathophysiologic risk perspective (heart failure development as a result of hyperlipidemia and atherosclerosis).

Finally, it is noteworthy that none of these studies addresses the expression levels of other AKAPs. This is important because several AKAPs are present in the heart. Thus, observed alterations that were accredited to AKAP12 may have been influenced by other AKAPs, especially AKAP5, as AKAP5 also binds to β_2-AR and is capable of switching the coupling of β_2-AR from the Gαs to the Gαi pathway.[128] In addition, AKAP5 can form not only homodimers but also a higher order supermolecular homo-oligomeric complex, thus influencing the stability of protein–protein interactions and signaling loops affecting the AKAP12 signalosome.[9] Therefore, these aspects stress the importance of taking into consideration the AKAP5 expression levels in future studies. Together, on the basis of the aforementioned data, AKAP12's role in the modulating cardiac function seems clearer. Thus, ongoing research of AKAP12's overexpression and how this overexpression impacts the cardiovascular system may resolve some of these issues.

In conclusion, AKAPs mediate the kinase signaling pathways needed for optimal cellular responses and alterations in their levels; either upregulation or downregulation explains the development or mitigation of different pathologic states. AKAP12 cellular signaling roles are established clearly in some systems (Figure 4).[99,102,129] However, the implication of this finding in cardiovascular diseases is still speculative, especially in the area of heart failure progression. Further understanding of AKAP12 binding domains and signaling pathways may be the initial step in developing specific peptide-targeting drugs.

ARTICLE INFORMATION

Affiliations

From the Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, Texas (H.Q., B.K.M.).

Acknowledgments

This work was done in partial fulfillment of the requirements for a PhD in pharmacology in the Department of Pharmacological and Pharmaceutical Sciences at the College of Pharmacy, University of Houston (H.Q.).

Sources of Funding

This study was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health (award number R15 HL141963 to B.K.M.); the American Heart Association (award number 18AIREA 33960175 to B.K.M.); and the Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation (to B.K.M.). The funders had no role in the preparation or decision to publish this work.

Disclosures

None.

REFERENCES

1. Langeberg LK, Scott JD. A-kinase-anchoring proteins. *J Cell Sci*. 2005;118:3217–3220.
2. Lohmann SM, DeCamilli P, Einig I, Walter U. High-affinity binding of the regulatory subunit (RII) of cAMP-dependent protein kinase to

microtubule-associated and other cellular proteins. *Proc Natl Acad Sci USA*. 1984;81:6723–6727.
3. Skroblin P, Grossmann S, Schäfer G, Rosenthal W, Klussmann E. Mechanisms of protein kinase A anchoring. In: Jeon K, ed. *Int Rev Cell Mol Biol*. Academic Press; 2010:235–330.
4. Newlon MG, Roy M, Morikis D, Hausken ZE, Coghlan V, Scott JD, Jennings PA. The molecular basis for protein kinase A anchoring revealed by solution NMR. *Nat Struct Biol*. 1999;6:222–227.
5. Fraser ID, Tavalin SJ, Lester LB, Langeberg LK, Westphal AM, Dean RA, Marrion NV, Scott JD. A novel lipid-anchored A-kinase anchoring protein facilitates cAMP-responsive membrane events. *EMBO J*. 1998;17:2261–2272.
6. Dell'Acqua ML, Faux MC, Thorburn J, Thorburn A, Scott JD. Membrane-targeting sequences on AKAP79 bind phosphatidylinositol-4, 5-bisphosphate. *EMBO J*. 1998;17:2246–2260.
7. Westphal RS, Soderling SH, Alto NM, Langeberg LK, Scott JD. Scar/WAVE-1, a Wiskott-Aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. *EMBO J*. 2000;19:4589–4600.
8. Colledge M, Scott JD. AKAPs: from structure to function. *Trends Cell Biol*. 1999;9:216–221.
9. Gao S, Wang HY, Malbon CC. AKAP5 and AKAP12 form homo-oligomers. *J Mol Signal*. 2011;6:3.
10. Smith FD, Langeberg LK, Scott JD. The where's and when's of kinase anchoring. *Trends Biochem Sci*. 2006;31:316–323.
11. Jarnaess E, Tasken K. Spatiotemporal control of camp signalling processes by anchored signalling complexes. *Biochem Soc Trans*. 2007;35:931–937.
12. Tasken K, Aandahl EM. Localized effects of cAMP mediated by distinct routes of protein kinase A. *Physiol Rev*. 2004;84:137–167.
13. Kim C, Cheng CY, Saldanha SA, Taylor SS. PKA-I holoenzyme structure reveals a mechanism for cAMP-dependent activation. *Cell*. 2007;130:1032–1043.
14. Means CK, Lygren B, Langeberg LK, Jain A, Dixon RE, Vega AL, Gold MG, Petrosyan S, Taylor SS, Murphy AN, et al. An entirely specific type i a-kinase anchoring protein that can sequester two molecules of protein kinase a at mitochondria. *Proc Natl Acad Sci*. 2011;108:E1227–E1235
15. Kim C, Xuong NH, Taylor SS. Crystal structure of a complex between the catalytic and regulatory (Rlalpha) subunits of PKA. *Science*. 2005;307:690–696.
16. Greenwald EC, Saucerman JJ. Bigger, better, faster: principles and models of AKAP anchoring protein signaling. *J Cardiovasc Pharmacol*. 2011;58:462–469.
17. Newlon MG, Roy M, Morikis D, Carr DW, Westphal R, Scott JD, Jennings PA. A novel mechanism of PKA anchoring revealed by solution structures of anchoring complexes. *EMBO J*. 2001;20:1651–1662.
18. Gold MG, Lygren B, Dokurno P, Hoshi N, McConnachie G, Tasken K, Carlson CR, Scott JD, Barford D. Molecular basis of AKAP specificity for PKA regulatory subunits. *Mol Cell*. 2006;24:383–395.
19. Kinderman FS, Kim C, von Daake S, Ma Y, Pham BQ, Spraggon G, Xuong N-H, Jennings PA, Taylor SS. A dynamic mechanism for akap binding to RII isoforms of cAMP-dependent protein kinase. *Mol Cell*. 2006;24:397–408.
20. Day ME, Gaietta GM, Sastri M, Koller A, Mackey MR, Scott JD, Perkins GA, Ellisman MH, Taylor SS. Isoform-specific targeting of pka to multivesicular bodies. *J Cell Biol.* 2011;193:347–363
21. Taylor SS, Kim C, Cheng CY, Brown SHJ, Wu J, Kannan N. Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design. *Biochim Biophysica Acta*. 2008;1784:16–26.
22. Stangherlin A, Zaccolo M. Local termination of 3'-5'-cyclic adenosine monophosphate signals: the role of a kinase anchoring protein-tethered phosphodiesterases. *J Cardiovasc Pharmacol*. 2011;58:345–353.
23. Carlisle Michel JJ, Dodge KL, Wong W, Mayer NC, Langeberg LK, Scott JD. PKA-phosphorylation of PDE4D3 facilitates recruitment of the mAKAP signalling complex. *Biochem J*. 2004;381:587–592.
24. Dell'Acqua ML, Dodge KL, Tavalin SJ, Scott JD. Mapping the protein phosphatase-2b anchoring site on AKAP79. Binding and inhibition of phosphatase activity are mediated by residues 315-360. *J Biol Chem*. 2002;277:48796–48802.
25. Dessauer CW. Adenylyl cyclase–A-kinase anchoring protein complexes: the next dimension in cAMP signaling. *Mol Pharmacol*. 2009;76:935–941.
26. Yan X, Walkiewicz M, Carlson J, Leiphon L, Grove B. Gravin dynamics regulates the subcellular distribution of PKA. *Exp Cell Res*. 2009;315:1247–1259.
27. Streb JW, Miano JM. Cross-species sequence analysis reveals multiple charged residue-rich domains that regulate nuclear/cytoplasmic partitioning and membrane localization of a kinase anchoring protein 12 (SSeCKS/gravin). *J Biol Chem*. 2005;280:28007–28014.
28. Lin F, Wang H, Malbon CC. Gravin-mediated formation of signaling complexes in beta 2-adrenergic receptor desensitization and resensitization. *J Biol Chem*. 2000;275:19025–19034.
29. Abi-Gerges A, Richter W, Lefebvre F, Mateo P, Varin A, Heymes C, Samuel J-L, Lugnier C, Conti M, Fischmeister R, Vandecasteele G. Decreased expression and activity of camp phosphodiesterases in cardiac hypertrophy and its impact on Beta-adrenergic camp signals. *Circ Res.* 2009;105:784–792
30. Lehnart SE, Wehrens XH, Reiken S, Warrier S, Belevych AE, Harvey RD, Richter W, Catherine Jin S-L, Conti M, Marks AR. Phosphodiesterase 4d deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. *Cell*. 2005;123:25–35
31. Lehnart SE, Marks AR. Phosphodiesterase 4D and heart failure: a cautionary tale. *Expert Opin Ther Targets*. 2006;10:677–688.
32. Weisenhaus M, Allen ML, Yang L, Lu Y, Nichols CB, Su T, Hell JW, McKnight GS. Mutations in AKAP5 disrupt dendritic signaling complexes and lead to electrophysiological and behavioral phenotypes in mice. *PLoS ONE*. 2010;5:e10325.
33. Jurado S, Biou V, Malenka RC. A calcineurin/AKAP complex is required for NMDA receptor-dependent long-term depression. *Nat Neurosci*. 2010;13:1053–1055.
34. Tunquist BJ, Hoshi N, Guire ES, Zhang F, Mullendorff K, Langeberg LK, Raber J, Scott JD. Loss of AKAP150 perturbs distinct neuronal processes in mice. *Proc Natl Acad Sci USA*. 2008;105:12557–12562.
35. Ruppelt A, Mosenden R, Gronholm M, Aandahl EM, Tobin D, Carlson CR, Abrahamsen H, Herberg FW, Carpén O, Taskén K. Inhibition of t cell activation by cyclic adenosine 5'-monophosphate requires lipid raft targeting of protein kinase a type i by the a-kinase anchoring protein ezrin. *J Immunol.* 2007;179:5159–5168
36. Lemay J, Maidou-Peindara P, Cancio R, Ennifar E, Coadou G, Maga G, Rain J-C, Benarous R, Liu LX. AKAP149 binds to HIV-1 reverse transcriptase and is involved in the reverse transcription. *J Mol Biol*. 2008;383:783–796.
37. Miki K, Willis WD, Brown PR, Goulding EH, Fulcher KD, Eddy EM. Targeted disruption of the AKAP4 gene causes defects in sperm flagellum and motility. *Dev Biol*. 2002;248:331–342.
38. Luconi M, Carloni V, Marra F, Ferruzzi P, Forti G, Baldi E. Increased phosphorylation of AKAP by inhibition of phosphatidylinositol 3-kinase enhances human sperm motility through tail recruitment of protein kinase A. *J Cell Sci*. 2004;117:1235–1246.
39. Newhall KJ, Criniti AR, Cheah CS, Smith KC, Kafer KE, Burkart AD, McKnight GS. Dynamic anchoring of PKA is essential during oocyte maturation. *Curr Biol*. 2006;16:321–327.
40. Lester LB, Faux MC, Nauert JB, Scott JD. Targeted protein kinase A and PP2B regulate insulin secretion through reversible phosphorylation. *Endocrinology*. 2001;142:1218–1227.
41. Josefsen K, Lee YC, Thams P, Efendic S, Nielsen JH. AKAP 18 alpha and gamma have opposing effects on insulin release in INS-1e cells. *FEBS Lett*. 2010;584:81–85.
42. Baltzer S, Klussmann E. Small molecules for modulating the localisation of the water channel aquaporin-2-disease relevance and perspectives for targeting local cAMP signalling. *Naunyn Schmiedebergs Arch Pharmacol*. 2019;392:1049–1064.
43. Schrade K, Troger J, Eldahshan A, Zuhlke K, Abdul Azeez KR, Elkins JM, Neuenschwander M, Oder A, Elkewedi M, Jaksch S, et al. An akap-lbc-rhoa interaction inhibitor promotes the translocation of aquaporin-2 to the plasma membrane of renal collecting duct principal cells. *PLoS One.* 2018;13:e0191423
44. Narala VR, Fukumoto J, Hernandez-Cuervo H, Patil SS, Krishnamurthy S, Breitzig M, Galam L, Soundararajan R, Lockey RF, Kolliputi N. Akap1 genetic deletion increases the severity of hyperoxia-induced acute lung injury in mice. *Am J Physiol Lung Cell Mol Physiol.* 2018;314:L860–L870
45. Xie G, Raufman JP. Association of protein kinase a with AKAP150 facilitates pepsinogen secretion from gastric chief cells. *Am J Physiol Gastrointest Liver Physiol*. 2001;281:G1051–1058.
46. Yu H, Zhou J, Takahashi H, Yao W, Suzuki Y, Yuan X, Yoshimura SH, Zhang Y, Liu Y, Emmett N. Spatial control of proton pump h,k-atpase

docking at the apical membrane by phosphorylation-coupled ezrin-syntaxin 3 interaction. *J Biol Chem.* 2014;289:33333–33342
47. Dukic AR, Haugen LH, Pidoux G, Leithe E, Bakke O, Tasken K. A protein kinase A–ezrin complex regulates Connexin 43 gap junction communication in liver epithelial cells. *Cell Signal*. 2017;32:1–11.
48. Lymperopoulos A, Rengo G, Koch WJ. Adrenergic nervous system in heart failure: pathophysiology and therapy. *Circ Res*. 2013;113:739–753.
49. Law NC, White MF, Hunzicker-Dunn ME. G protein-coupled receptors (GPCRs) that signal via protein kinase A (PKA) cross-talk at insulin receptor substrate 1 (IRS1) to activate the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. *J Biol Chem*. 2016;291:27160–27169.
50. Rich TC, Fagan KA, Tse TE, Schaack J, Cooper DM, Karpen JW. A uniform extracellular stimulus triggers distinct cAMP signals in different compartments of a simple cell. *Proc Natl Acad Sci USA*. 2001;98:13049–13054.
51. Seino S, Shibasaki T. PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. *Physiol Rev*. 2005;85:1303–1342.
52. Houslay MD, Baillie GS, Maurice DH. CAMP-specific phosphodiesterase-4 enzymes in the cardiovascular system: a molecular toolbox for generating compartmentalized cAMP signaling. *Circ Res*. 2007;100:950–966.
53. Diviani D, Osman H, Delaunay M, Kaiser S. The role of A-kinase anchoring proteins in cardiac oxidative stress. *Biochem Soc Trans*. 2019;47:1341–1353.
54. Wong W, Goehring AS, Kapiloff MS, Langeberg LK, Scott JD. mAKAP compartmentalizes oxygen-dependent control of HIF-1α. *Sci Signaling*. 2008;1:ra18-ra18.
55. Lygren B, Carlson CR, Santamaria K, Lissandron V, McSorley T, Litzenberg J, Lorenz D, Wiesner B, Rosenthal W, Zaccolo M et al. Akap complex regulates ca2+ re-uptake into heart sarcoplasmic reticulum. *EMBO Rep.* 2007;8:1061–1067
56. Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS. Requirement of a macromolecular signaling complex for β adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science*. 2002;295:496–499.
57. Tingley WG, Pawlikowska L, Zaroff JG, Kim T, Nguyen T, Young SG, Vranizan K, Kwok P-Y, Whooley MA, Conklin BR. Gene-trapped mouse embryonic stem cell-derived cardiac myocytes and human genetics implicate akap10 in heart rhythm regulation. *Proc Natl Acad Sci U S A*. 2007;104:8461–8466
58. Guillory AN, Yin X, Wijaya CS, Diaz Diaz AC, Rababa'h A, Singh S, Atrooz F, Sadayappan S, McConnell BK. Enhanced cardiac function in gravin mutant mice involves alterations in the beta-adrenergic receptor signaling cascade. *PLoS ONE*. 2013;8:e74784
59. Li Z, Singh S, Suryavanshi SV, Ding W, Shen X, Wijaya CS, Gao WD, McConnell BK. Force development and intracellular Ca(2+) in intact cardiac muscles from gravin mutant mice. *Eur J Pharmacol*. 2017;807:117–126.
60. Li Y, Yu QH, Chu Y, Wu WM, Song JX, Zhu XB, Wang Q. Blockage of AKAP12 accelerates angiotensin II (Ang II)-induced cardiac injury in mice by regulating the transforming growth factor beta1 (TGF-beta1) pathway. *Biochem Biophys Res Commun*. 2018;499:128–135.
61. Fan Q, Yin X, Rababa'h A, Diaz Diaz A, Wijaya CS, Singh S, Suryavanshi SV, Vo HH, Saeed M, Zhang Yet al. Absence of gravin-mediated signaling inhibits development of high-fat diet-induced hyperlipidemia and atherosclerosis. *Am J Physiol Heart Circ Physiol.* 2019;317:H793–H810
62. Johnson KR, Nicodemus-Johnson J, Spindler MJ, Carnegie GK. Genome-wide gene expression analysis shows AKAP13-mediated PKD1 signaling regulates the transcriptional response to cardiac hypertrophy. *PLoS ONE*. 2015;10:e0132474.
63. Garcia-Pelagio KP, Chen L, Joca HC, Ward C, Jonathan Lederer W, Bloch RJ. Absence of synemin in mice causes structural and functional abnormalities in heart. *J Mol Cell Cardiol*. 2018;114:354–363.
64. Benson MA, Tinsley CL, Waite AJ, Carlisle FA, Sweet SMM, Ehler E, George CH,Lai FA, Martin-Rendon E, Blake DJ. Ryanodine receptors are part of the myospryn complex in cardiac muscle. *Sci Rep.* 2017;7:6312
65. Mayers CM, Wadell J, McLean K, Venere M, Malik M, Shibata T, Driggers PH, Kino T, Guo XC, Koide H, et al. The rho guanine nucleotide exchange factor akap13 (brx) is essential for cardiac development in mice. *J Biol Chem.* 2010;285:12344–12354
66. Passariello CL, Li J, Dodge-Kafka K, Kapiloff MS. mAKAP—a master scaffold for cardiac remodeling. *J Cardiovasc Pharmacol*. 2015;65:218–225.
67. Kapiloff MS, Jackson N, Airhart N. mAKAP and the ryanodine receptor are part of a multi-component signaling complex on the cardiomyocyte nuclear envelope. *J Cell Sci*. 2001;114:3167–3176.
68. Kapiloff MS, Piggott LA, Sadana R, Li J, Heredia LA, Henson E, Efendiev R, Dessauer CW. An adenylyl cyclase-mAKAPbeta signaling complex regulates camp levels in cardiac myocytes. *J Biol Chem*. 2009;284:23540–23546.
69. Dodge-Kafka KL, Soughayer J, Pare GC, Carlisle Michel JJ, Langeberg LK, Kapiloff MS, Scott JD. The protein kinase A anchoring protein mAKAP coordinates two integrated camp effector pathways. *Nature*. 2005;437:574–578.
70. Dodge KL, Khouangsathiene S, Kapiloff MS, Mouton R, Hill EV, Houslay MD, Langeberg LK, Scott JD. mAKAP assembles a protein kinase A/PDE4 phosphodiesterase camp signaling module. *EMBO J*. 2001;20:1921–1930.
71. Marx SO, Reiken S, Hisamatsu Y, Gaburjakova M, Gaburjakova J, Yang YM, Rosemblit N, Marks AR. Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. *J Cell Biol*. 2001;153:699–708.
72. McCartney S, Little BM, Langeberg LK, Scott JD. Cloning and characterization of A-kinase anchor protein 100 (AKAP100). A protein that targets A-kinase to the sarcoplasmic reticulum. *J Biol Chem*. 1995;270:9327–9333.
73. Yang J, Drazba JA, Ferguson DG, Bond M. A-kinase anchoring protein 100 (AKAP100) is localized in multiple subcellular compartments in the adult rat heart. *J Cell Biol*. 1998;142:511–522.
74. Kapiloff MS, Jackson N, Airhart N. mAKAP and the ryanodine receptor are part of a multi-component signaling complex on the cardiomyocyte nuclear envelope. *J Cell Sci*. 2001;114:3167–3176.
75. Zhang L, Malik S, Kelley GG, Kapiloff MS, Smrcka AV. Phospholipase C epsilon scaffolds to muscle-specific a kinase anchoring protein (mAKAPbeta) and integrates multiple hypertrophic stimuli in cardiac myocytes. *J Biol Chem*. 2011;286:23012–23021.
76. Rababa'h A, Singh S, Suryavanshi SV, Altarabsheh SE, Deo SV, McConnell BK. Compartmentalization role of a-kinase anchoring proteins (akaps) in mediating protein kinase a (pka) signaling and cardiomyocyte hypertrophy. *Int J Mol Sci* 2014;16:218–229.
77. Russell MA, Lund LM, Haber R, McKeegan K, Cianciola N, Bond M. The intermediate filament protein, synemin, is an AKAP in the heart. *Arch Biochem Biophys*. 2006;456:204–215.
78. Uys GM, Ramburan A, Loos B, Kinnear CJ, Korkie LJ, Mouton J, Riedemann J, Moolman-Smook JC. Myomegalin is a novel A-kinase anchoring protein involved in the phosphorylation of cardiac myosin binding protein C. *BMC Cell Biol*. 2011;12:18.
79. Cheng EP, Yuan C, Navedo MF, Dixon RE, Nieves-Cintron M, Scott JD, Santana LF. Restoration of normal l-type Ca^{2+} channel function during Timothy syndrome by ablation of an anchoring protein. *Circ Res*. 2011;109:255–261.
80. Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Marks AR, Kass RS. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science*. 2002;295:496–499.
81. Crabtree GR, Olson EN. NFAT signaling: choreographing the social lives of cells. *Cell*. 2002;109(Suppl):S67–79.
82. Vega RB, Harrison BC, Meadows E, Roberts CR, Papst PJ, Olson EN, McKinsey TA. Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. *Mol Cell Biol*. 2004;24:8374–8385.
83. Aye TT, Soni S, van Veen TA, van der Heyden MA, Cappadona S, Varro A, de Weger RA, de Jonge N, Vos MA, Heck AJR, Scholten Al. Reorganized pka-akap associations in the failing human heart. *J Mol Cell Cardiol.* 2012;52:511–518
84. Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, Gorelik J. Beta2-adrenergic receptor redistribution in heart failure changes camp compartmentation. *Science*. 2010;327:1653–1657
85. Perino A, Ghigo A, Scott JD, Hirsch E. Anchoring proteins as regulators of signaling pathways. *Circ Res*. 2012;111:482–492.
86. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. *Nature*. 2002;415:206–212.
87. Xiang Y, Rybin VO, Steinberg SF, Kobilka B. Caveolar localization dictates physiologic signaling of beta 2-adrenoceptors in neonatal cardiac myocytes. *J Biol Chem*. 2002;277:34280–34286.
88. Rajagopal S, Rajagopal K, Lefkowitz RJ. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat Rev Drug Discov*. 2010;9:373–386.

89. Violin JD, DiPilato LM, Yildirim N, Elston TC, Zhang J, Lefkowitz RJ. Beta2-adrenergic receptor signaling and desensitization elucidated by quantitative modeling of real time cAMP dynamics. *J Biol Chem*. 2008;283:2949–2961.
90. Penn RB, Panettieri RA Jr, Benovic JL. Mechanisms of acute desensitization of the beta2AR-adenylyl cyclase pathway in human airway smooth muscle. *Am J Respir Cell Mol Biol*. 1998;19:338–348.
91. Nino G, Hu A, Grunstein JS, Grunstein MM. Mechanism regulating proasthmatic effects of prolonged homologous beta2-adrenergic receptor desensitization in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol*. 2009;297:L746–757.
92. Goral V, Jin Y, Sun H, Ferrie AM, Wu Q, Fang Y. Agonist-directed desensitization of the beta2-adrenergic receptor. *PLoS ONE*. 2011;6:e19282.
93. Fan X, Gu X, Zhao R, Zheng Q, Li L, Yang W, Ding L, Xue F, Fan J, Gong Y, Wang Y. Cardiac beta2-adrenergic receptor phosphorylation at ser355/356 regulates receptor internalization and functional resensitization. *PLoS One.* 2016;11:e0161373
94. Uchida Y, Rutaganira FU, Jullie D, Shokat KM, von Zastrow M. Endosomal phosphatidylinositol 3-kinase is essential for canonical GPCR signaling. *Mol Pharmacol*. 2017;91:65–73.
95. Kim H, Scimia MC, Wilkinson D, Trelles RD, Wood MR, Bowtell D, Dillin A, Mercola M, Ronai Z. Fine-tuning of drp1/fis1 availability by akap121/siah2 regulates mitochondrial adaptation to hypoxia. *Mol Cell.* 2011;44:532–544
96. Ruehr ML, Russell MA, Bond M. A-kinase anchoring protein targeting of protein kinase A in the heart. *J Mol Cell Cardiol*. 2004;37:653–665.
97. Gordon T, Grove B, Loftus JC, O'Toole T, McMillan R, Lindstrom J, Ginsberg MH. Molecular cloning and preliminary characterization of a novel cytoplasmic antigen recognized by myasthenia gravis sera. *J Clin Invest*. 1992;90:992–999.
98. Xia W, Unger P, Miller L, Nelson J, Gelman IH. The Src-suppressed C kinase substrate, SSeCKS, is a potential metastasis inhibitor in prostate cancer. *Cancer Res*. 2001;61:5644–5651.
99. Piontek J, Brandt R. Differential and regulated binding of cAMP-dependent protein kinase and protein kinase C isoenzymes to Gravin in human model neurons: evidence that gravin provides a dynamic platform for the localization for kinases during neuronal development. *J Biol Chem*. 2003;278:38970–38979.
100. Tao J, Shumay E, McLaughlin S, Wang HY, Malbon CC. Regulation of AKAP-membrane interactions by calcium. *J Biol Chem*. 2006;281:23932–23944.
101. Tao J, Wang HY, Malbon CC. Protein kinase A regulates AKAP250 (Gravin) scaffold binding to the beta2-adrenergic receptor. *EMBO J*. 2003;22:6419–6429.
102. Akakura S, Gelman IH. Pivotal role of AKAP12 in the regulation of cellular adhesion dynamics: control of cytoskeletal architecture, cell migration, and mitogenic signaling. *J Signal Transduction*. 2012;2012:529179.
103. Gelman IH. Emerging roles for SSeCKS/Gravin/AKAP12 in the control of cell proliferation, cancer malignancy, and barriergenesis. *Genes Cancer*. 2010;1:1147–1156.
104. Gelman IH. The role of SSeCKS/Gravin/AKAP12 scaffolding proteins in the spatiotemporal control of signaling pathways in oncogenesis and development. *Front Biosci*. 2002;7:d1782–1797.
105. Streb JW, Kitchen CM, Gelman IH, Miano JM. Multiple promoters direct expression of three AKAP12 isoforms with distinct subcellular and tissue distribution profiles. *J Biol Chem*. 2004;279:56014–56023.
106. Muramatsu M, Gao L, Peresie J, Balderman B, Akakura S, Gelman IH. SSeCKS/AKAP12 scaffolding functions suppress B16F10-induced peritoneal metastasis by attenuating CXCL9/10 secretion by resident fibroblasts. *Oncotarget*. 2017;8:70281–70298.
107. Reggi E, Diviani D. The role of A-kinase anchoring proteins in cancer development. *Cell Signal*. 2017;40:143–155.
108. Gelman IH. Suppression of tumor and metastasis progression through the scaffolding functions of SSeCKS/Gravin/AKAP12. *Cancer Metastasis Rev*. 2012;31:493–500.
109. Alshabi AM, Vastrad B, Shaikh IA. Vastrad C. Identification of crucial candidate genes and pathways in glioblastoma multiform by bioinformatics analysis. *Biomolecules*. 2019;9:201.
110. Li Z, Hu J, Guo J, Fan L, Wang S, Dou N, Zuo J, Yu S. SSeCKS/Gravin/AKAP12 inhibits PKCzeta-mediated reduction of ERK5 transactivation to prevent endotoxin-induced vascular dysfunction. *Cardiovasc Toxicol*. 2019;19:372–381.
111. Wei H, Xu L, Li C, Liu L, Ng DM, Haleem M, Jiang L,Sun N, Ling Q, Ma S, et al. Ssecks promoted lipopolysaccharide-sensitized astrocytes migration via increasing beta-1,4-galactosyltransferase-i activity. *Neurochem Res.* 2019;44:839–848
112. Wu X, Wu T, Li K, Li Y, Hu TT, Wang WF, Qiang SJ, Xue SB, Liu WW. The mechanism and influence of akap12 in different cancers. *Biomed Environ Sci.* 2018;31:927–932
113. Cao Z, Singh B, Li C, Markham NO, Carrington LJ, Franklin JL, et al Protein kinase A-mediated phosphorylation of naked cuticle homolog 2 stimulates cell-surface delivery of transforming growth factor-alpha for epidermal growth factor receptor transactivation. *Traffic*. 2019;20:357–368.
114. Billington CK, Penn RB. Signaling and regulation of G protein-coupled receptors in airway smooth muscle. *Respir Res*. 2003;4:2.
115. Malbon CC, Tao J, Wang HY. AKAPs (A-kinase anchoring proteins) and molecules that compose their G-protein-coupled receptor signalling complexes. *Biochem J*. 2004;379:1–9.
116. Fan G, Shumay E, Wang H, Malbon CC. The scaffold protein Gravin (cAMP-dependent protein kinase-anchoring protein 250) binds the beta 2-adrenergic receptor via the receptor cytoplasmic Arg-329 to Leu-413 domain and provides a mobile scaffold during desensitization. *J Biol Chem*. 2001;276:24005–24014.
117. Tao J, Wang HY, Malbon CC. AKAR2-AKAP12 fusion protein "biosenses" dynamic phosphorylation and localization of a GPCR-based scaffold. *J Mol Signal*. 2010;5:3.
118. Chen MH, Malbon CC. G-protein-coupled receptor-associated A-kinase anchoring proteins AKAP5 and AKAP12: differential trafficking and distribution. *Cell Signal*. 2009;21:136–142.
119. Cong M, Perry SJ, Lin FT, Fraser ID, Hu LA, Chen W, Pitcher JA, Scott JD, Lefkowitz RJ. Regulation of membrane targeting of the g protein-coupled receptor kinase 2 by protein kinase a and its anchoring protein akap79. *J Biol Chem.* 2001;276:15192–15199
120. Radeva MY, Kugelmann D, Spindler V, Waschke J. Pka compartmentalization via AKAP220 and AKAP12 contributes to endothelial barrier regulation. *PLoS ONE*. 2014;9:e106733.
121. Kwon HB, Choi YK, Lim JJ, Kwon SH, Her S, Kim HJ, Lim K-J, Ahn J-C, Kim Y-M, Bae M-K, et al. Akap12 regulates vascular integrity in zebrafish. *Exp Mol Med.* 2012;44:225–235
122. Oldenburger A, Poppinga WJ, Kos F, de Bruin HG, Rijks WF, Heijink IH, Timens W, Meurs H, Maarsingh H, Schmidt M, et al. A-kinase anchoring proteins contribute to loss of e-cadherin and bronchial epithelial barrier by cigarette smoke. *Am J Physiol Cell Physiol.* 2014;306:C585–597
123. Weissmuller T, Glover LE, Fennimore B, Curtis VF, MacManus CF, Ehrentraut SF, Ehrentraut SF, Campbell EL, Scully M, Grove BD, et al. Hif-dependent regulation of akap12 (gravin) in the control of human vascular endothelial function. *FASEB J.* 2014;28:256–264
124. Binder AK, Rodriguez KF, Hamilton KJ, Stockton PS, Reed CE, Korach KS. The absence of ER-beta results in altered gene expression in ovarian granulosa cells isolated from in vivo preovulatory follicles. *Endocrinology*. 2013;154:2174–2187.
125. Adluri RS, Thirunavukkarasu M, Zhan L, Akita Y, Samuel SM, Otani H, Ho YS, Maulik G, Maulik N, et al. Thioredoxin 1 enhances neovascularization and reduces ventricular remodeling during chronic myocardial infarction: A study using thioredoxin 1 transgenic mice. *J Mol Cell Cardiol.* 2011;50:239–247
126. Choi YK, Kim JH, Kim WJ, Lee HY, Park JA, Lee SW, Yoon D-K, Kim HH, Chung H, Yu YS, et al. Akap12 regulates human blood-retinal barrier formation by downregulation of hypoxia-inducible factor-1alpha. *J Neurosci.* 2007;27:4472–4481
127. Choi MC, Lee YU, Kim SH, Lee JH, Park JH, Streb JW, Oh D-Y, Im S-A, Kim T-Y, Jong H-S, et al. Overexpression of a-kinase anchoring protein 12a activates sterol regulatory element binding protein-2 and enhances cholesterol efflux in hepatic cells. *Int J Biochem Cell Biol.* 2008;40:2534–2543
128. Poppinga WJ, Munoz-Llancao P, Gonzalez-Billault C, Schmidt M. A-kinase anchoring proteins: cAMP compartmentalization in neurodegenerative and obstructive pulmonary diseases. *Br J Pharmacol*. 2014;171:5603–5623.
129. Su B, Bu Y, Engelberg D, Gelman IH. SSeCKS/Gravin/AKAP12 inhibits cancer cell invasiveness and chemotaxis by suppressing a protein kinase C–Raf/MEK/ERK pathway. *J Biol Chem*. 2010;285:4578–4586.