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Review Article

The role of A-kinase anchoring proteins in cardiac oxidative stress

Dario Diviani, Halima Osman, Marion Delaunay and Simon Kaiser

Département de Pharmacologie et de Toxicologie, Faculté de Biologie et de Médecine, Lausanne 1011, Switzerland

Correspondence: Dario Diviani (Dario.diviani@unil.ch)

Cardiac stress initiates a pathological remodeling process that is associated with cardiomyocyte loss and fibrosis that ultimately leads to heart failure. In the injured heart, a pathologically elevated synthesis of reactive oxygen species (ROS) is the main driver of oxidative stress and consequent cardiomyocyte dysfunction and death. In this context, the cAMP-dependent protein kinase (PKA) plays a central role in regulating signaling pathways that protect the heart against ROS-induced cardiac damage. In cardiac cells, spatiotemporal regulation of PKA activity is controlled by A-kinase anchoring proteins (AKAPs). This family of scaffolding proteins tether PKA and other transduction enzymes at subcellular microdomains where they can co-ordinate cellular responses regulating oxidative stress. In this review, we will discuss recent literature illustrating the role of PKA and AKAPs in modulating the detrimental impact of ROS production on cardiac function.

Introduction

Mechanisms involved in reactive oxygen species generation in the heart

Under physiological conditions, redox signaling, defined as the reversible oxidation/reduction modification of cellular components, regulates the activity of transduction pathways influencing fundamental cardiac functions including excitation–contraction coupling, adaptation to oxygen availability, cardiac development and physiological hypertrophy [1].

In response to biomechanical and neurohumoral stresses, ischemia/reperfusion as well as exposure to drugs and toxicants the synthetic rate of oxidizing molecules such as reactive oxygen species (ROS) is pathologically increased and exceeds the endogenous detoxifying capacity of myocardial cells. This results in local oxidative stress, and subsequent cardiac damage [2–4].

The main sources of intracardiac ROS are the electron transport chain, nicotinamide adenine dinucleotide (NADPH) oxidaxes (Noxs), uncoupled nitric oxide (NO) synthases and xanthine oxidases (e.g. monoamine oxidases), whereas ROS detoxification involves antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase [1].

ROS including the superoxide anion (O_2^-), hydroxyl ions or radicals ($^{\cdot}OH$), peroxide (O_2^{-2}) and hydrogen peroxide (H_2O_2) can react with and oxidize various cellular targets in cardiomyocytes including signaling proteins, contractile and structural proteins, ion transporters, lipids and nucleic acids. This profoundly alters the function and viability of cardiac cells, which favors pathological remodeling and the development of heart failure [1,4].

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Mechanisms involved in ROS-mediated cardiac dysfunction

ROS-mediated oxidation events promote cardiac dysfunction by triggering multiple pathophysiological responses, which impair cardiomyocyte contractility and favor cardiac arrhythmia, promote cardiomyocyte hypertrophy and apoptosis, and induce cardiac fibrosis.

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Depressed contractile function and arrhythmia are the consequence of the severe dysregulation of calcium cycling induced by the oxidation of ryanodine receptors (RyRs) and the sarcoplasmic reticulum calcium ATPase (SERCA2) [5]. Additional direct effects of ROS on the sarcomeric protein titin have also been suggested to impact cardiac contractility.

ROS-induced cardiomyocyte apoptosis is triggered by DNA oxidation and damage and subsequent activation of p53-dependent apoptotic pathways, by the activation of the apoptosis signaling kinase-1 (ASK-1) or by the opening of the mitochondrial permeability transition complex (mPTC), which triggers further ROS release from mitochondria [1,6]. These events eventually result in the inhibition of the anti-apoptotic protein Bcl2 and the activation of pro-apoptotic proteins such as the Bcl2-associated X protein (Bax) and Bcl2-associated agonist of cell death (Bad). Activated Bax translocates from the cytosol to the mitochondria where it induces outer membrane permeabilization, which leads to the release of cytochrome C into the cytosol and the activation of pro-apoptotic caspases.

Studies performed in isolated cardiomyocytes and knockout (KO) animal models indicate that Nox2-dependent ROS production can favor cardiomyocyte hypertrophy and dysfunction in response to various stressors including Angiotensin II (Ang-II), endothelin 1 and pressure overload [7–10]. In this context, hypertrophy and heart failure have been proposed to involve ROS-dependent activation of pathways involving extracellular signal-regulated kinases (ERKs), p38 mitogen-activated protein kinases (MAPK), ASK-1 and nuclear factor-κB (NF-κB) [7,9,11,12].

Finally, generation of ROS in cardiac fibroblasts following Nox2 activation activates a gene program involved in the differentiation of fibroblasts into activated myofibroblasts, which secrete a large amount of extracellular matrix and promote cardiac fibrosis [7,13]. ROS also trigger fibrosis by inducing the transcriptional activation of pro-fibrotic ligands such as endothelin 1 or the connective tissue growth factor (CTGF) [13,14]. Importantly, ROS-mediated fibrosis negatively impacts cardiac function by impairing diastolic function and increasing the probability of developing arrhythmias.

Several clinical trials failed to show a beneficial effect of general antioxidant treatments in reducing cardiovascular morbidity and mortality. Based on these findings, the advantage of more specific approaches targeting selected Nox enzymes is now being investigated [7]. One alternative strategy that appears to efficiently reduce the detrimental effects of oxidative stress is the targeted activation of protective pathways that preserve cardiomyocyte function and viability. In this context, evidence collected over the last few years indicates that the cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) exerts a protective effect on ROS-mediated cardiac damage [15]. In particular, it appears that subcellular compartmentalization of PKA signaling through A-kinase anchoring proteins (AKAPs) is crucial in determining the specificity and the efficacy of the cytoprotective action of the kinase [16]. The current review will highlight the role of PKA signaling and AKAPs in cardiac protection against oxidative stress. The accent will be placed on molecular and pathophysiological events regulated by AKAP-based signaling complexes in stressed cardiac cells.

The role of PKA signaling in cardiac oxidative stress

PKA is a cAMP-activated serine/threonine kinase controlling numerous myocardial functions including Ca^{2+} cycling, contractility, action potential duration and adaptive responses to various cardiac stresses [17]. It is composed of a regulatory (R) subunit dimer and two catalytic (C) subunits. cAMP is synthesized by adenylyl cyclases in response to hormonal stimulation of Gs-coupled receptors expressed at the cell surface. It binds to two sites on each R subunits, which favors the activation of the C subunits. While it was generally assumed that this activation process requires the physical dissociation of the C subunits from the R subunits, recent findings now indicate that the heterodimer remains intact [18]. PKA can be classified as type I or type II based on the nature of the R subunit composition (RI or RII) of the holoenzyme. The two PKA types display different subcellular localization, PKA I being mainly (but not exclusively) cytosolic and PKA II predominantly associated with cell membranes and cytoskeleton [19]. Because of their differential compartmentalization, PKA I and PKA II regulate non-redundant functions within cardiac cells.

Accumulating evidence indicates that PKA I is a redox-regulated kinase that can be activated in a cAMP-independent manner through the oxidation of cysteine 17 located within the N-terminal region of RI subunits [20]. Cysteine oxidation induces the formation of an intersubunit disulfide bridge, which favors a RI subunit dimer conformation that displays an increased affinity for AKAPs [20,21]. This mechanism is proposed to enhance the targeting of PKA I to specific cellular substrates. Recent findings now indicate that activation of PKA I by oxidation promotes adaptive responses that protect the myocardium from oxidative stress-induced

cardiac dysfunction. This concept is illustrated by a study showing that knock-in (KI) mice carrying a cysteine 17 to serine mutation in the RIα gene display a strong decrease in the phosphorylation of the Cav1.2 calcium channel at the PKA site when subjected to thoracic aortic constriction (TAC)-induced pressure overload or Ang-II infusion [15]. Reduced phosphorylation levels negatively impact Cav1.2 Ca^{2+} current and Ca^{2+} transient amplitude, and, as a consequence, decrease ejection fraction (EF), cause a prolongation of the QT interval, and increase the propensity for ventricular arrhythmias [15]. Therefore, oxidative modification of PKA in response to stimuli that promote cardiac oxidative stress represents a compensatory response that is crucial to maintain Ca^{2+} signaling and contractility.

Many studies indicate that activation of PKA, achieved through direct stimulation of ACs with forskolin, inhibition of phosphodiesterases 3 and 4, or induced by receptor ligands such as adiponectin, the glucagon-like peptide 1 (GLP1) agonist exendin 4 and adrenomedullin, can favor cardiomyocyte protection against a variety of oxidative stress-inducing stimuli including ischemia/reperfusion (I/R), hyperglycemia, anthracycline exposure, pressure overload, or H_2O_2 [22–29]. In this context, many mechanisms have been shown to mediate the protective effects of PKA, which include inhibition of nuclear factor Kappa B (NF-κB) signaling [23,26], decrease in Nox expression and anion superoxide production [29], inhibition of pro-apoptotic Bcl2-associated agonist of cell death (Bad) protein expression [22] and reduction in mitochondrial ROS levels [30].

In line with the notion that the cAMP/PKA pathway confers cardioprotection, recent findings indicate that oxidative stress can induce cell death by negatively regulating PKA signaling. In this respect, it appears that ROS can alter the mitochondrial structure and promote H9c2 myoblast apoptosis by decreasing mitochondrial cAMP [31]. The consequent reduction in PKA activity has been shown to cause degradation of the deacetylase sirtuin3 (Sirt3) and, in turn, promote hyperacetylation and proteolytic processing of optic atrophy 1 (OPA1) a GTPase that controls mitochondrial fusion and integrity [31].

Collectively, these findings highlight a key role for PKA in integrating redox signaling and in attenuating the detrimental impact of oxidative stress in cardiomyocytes.

AKAP-mediated regulation of cardiac oxidative stress

AKAPs constitute a family of scaffolding proteins involved in the compartmentalization of PKA and other signaling enzymes within cellular microdomains [19]. By organizing macromolecular signaling complexes at discrete cellular sites in proximity of activators and downstream effectors, AKAPs specify spatiotemporal regulation of transduction pathways and cellular responses [32]. PKA anchoring is achieved through a conserved amphipathic helix, which directly interacts with a hydrophobic region located in the dimerization/docking (D/D) domain of the R subunit. While most AKAPs bind RII subunits, some dual-specificity AKAPs have been described that anchor both RI and RII isoforms [19].

AKAPs anchor and co-ordinate the activity of diverse signaling enzymes such as kinases, phosphatases, adenylyl cyclases, phosphodiesterases, or GTPases [16,33]. The assembly of such multivalent signalosomes allows AKAPs to integrate and co-ordinate multiple pathways cells and to ensure the precise modulation of sophisticated cellular responses.

Evidence accumulated over the last two decades indicates that AKAPs regulate several key cardiac functions including Ca^{2+} cycling and contraction, action potential propagation and heart rhythm, and adaptive responses to various cardiac stresses and insults. In line with these observations, alterations in AKAP signaling or expression in heart cells have been shown to be linked to pathologies such as arrhythmias, cardiac remodeling and heart failure [16,34].

Importantly, it has recently emerged that AKAPs are crucially involved in regulating pathological responses associated with cardiac oxidative stress. On the one hand, this family of anchoring proteins can modulate ROS production in cardiomyocytes and in cardiac fibroblasts by influencing mitochondria biogenesis [35], mitochondrial dynamics [36,37] and/or by directly regulating signaling pathways involved in ROS generation or detoxification [38]. On the other hand, AKAPs can also modulate the ability of ROS to activate cardiomyocyte apoptosis and fibrosis, two major inducers of heart damage and failure.

In the following sections, we will discuss recent literature illustrating the complex interplay between AKAPs and cardiac oxidative stress, and highlight the key role of AKAP-based transduction complexes in modulating ROS formation and ROS-induced myocardial dysfunction. In particular, we will focus on four anchoring proteins including AKAP1, AKAP5, AKAP12 and AKAP13, which have been shown to directly affect oxidative responses in cardiac cells. For more information about the role of AKAPs in regulating homeostatic and adaptive functions in the cardiovascular system, we refer the reader to recent reviews [16,33,34,39,40].

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AKAP1/D-AKAP1

AKAP1 (or D-AKAP1, AKAP121, AKAP149) localizes to the outer membrane of mitochondria where it regulates multiple functions including oxidative phosphorylation, ATP production and mitochondrial Ca^{2+} homeostasis [41,42]. The anchoring protein also promotes cardioprotection by inhibiting mitochondrial fission (fragmentation) and maintaining the integrity of mitochondria.

Mitochondrial fission is typically observed in hypoxic or stressed cardiomyocytes and is known to directly alter the morphology and impair the activity of mitochondria, resulting in enhanced ROS production and increased cardiomyocyte apoptosis [43,44]. Fission is mediated by the interaction between a GTPase named dynamin-related protein 1 (DRP1) and the mitochondrial fission protein 1 (FIS1) located at the outer membrane of mitochondria [44,45]. Fis-mediated recruitment of DRP1 at the mitochondrial surface favors local GTP hydrolysis, which provides the driving force for fission [44,45].

In this context, AKAP1 modulates the function of DRP1 and FIS1 in at least two ways. Firstly, AKAP1-anchored PKA directly phosphorylates DRP1 at serine 637, inducing its detachment from the mitochondrial membrane (Figure 1) [37]. Secondly, AKAP1 physically inhibits the formation of the DRPI/FIS1 complex, thus inhibiting mitochondrial fission and cardiomyocyte apoptosis (Figure 1) [37].

Several studies indicate that AKAP1 is rapidly proteolyzed in response to various cardiac stresses or insults including hypoxia, biomechanical stress and myocardial lipid accumulation [36,37,46–48]. The mechanisms involved in AKAP1 down-regulation have been characterized both *in vitro* and *in vivo* in mouse models subjected to cardiac ischemia or pressure overload. They involve the activation of the ubiquitin-protein ligase seven in absentia homolog 2 (SIAH2), which ubiquitylates and promotes proteasomal degradation of AKAP1 (Figure 1) [37,46,47].

AKAP1 down-regulation decreases phosphorylation of DRP1 by PKA, which, in turn, favors the formation of stable FIS1/DRPI complexes and mitochondrial fission. This is associated with increased ROS production inside mitochondria, oxidative stress and cardiac dysfunction (Figure 1) [36,37].

The mechanisms whereby the reduction in AKAP1 levels promotes oxidative stress in cardiomyocytes have not been fully elucidated. One possibility is that increased mitochondrial fission impacts the flux of electrons through the electron transport chain, which would favor side reactions giving rise to O_2^- and subsequent ROS production [1,49]. Alternatively, based on previous findings showing that AKAP1 enhances the recruitment of the superoxide dismutase 2 (SOD2) mRNA to mitochondria and mitochondrial SOD2 expression, one could speculate that reduced AKAP1 expression could decrease mitochondrial SOD2 content, which would impair O_2^- clearing, and increase ROS production [38] (Figure 1).

Collectively, the findings illustrated above strongly suggest that AKAP1 is a cardioprotective protein that maintains mitochondrial function, reduces oxidative stress and prevents cardiomyocyte death. In this context, prevention of apoptosis could be linked to the inhibitory effect of AKAP1 on mitochondrial fission and ROS production, or, as suggested by *in vitro* studies, to the ability of AKAP1 to promote PKA-dependent phosphorylation and inhibition of the pro-apoptotic Bad and subsequent cytochrome C release from mitochondria [50] (Figure 1). Therefore, AKAP1 down-regulation in stressed hearts could be viewed as a detrimental event leading to mitochondrial dysfunction and heart failure.

AKAP5/AKAP79

AKAP5 (or AKAP79/150) was originally isolated as an RII-binding protein from a human thyroid expression library. Later studies showed that this anchoring is expressed in neurons, cardiomyocytes and other cell types, where it localizes at the inner side of the plasma membrane. At this subcellular location, it anchors several signaling molecules including PKA, PKC and the phosphatase calcineurin [19,51,52] in the proximity of upstream activators.

In cardiomyocytes, AKAP5 has been shown co-ordinate pathophysiological signals involved in glucotoxicity [53]. Chronic hyperglycemia is known to trigger cardiac complications including systolic and diastolic alterations, fibrosis and hypertrophy, which are associated with morbidity and mortality in diabetic patients [54]. Recent evidence indicates that hyperglycemia transcriptionally up-regulated AKAP5 in cardiomyocytes. This was observed both *in vivo*, in streptozotocin-induced diabetic rats, and *in vitro*, in neonatal rat cardiomyocytes [53]. AKAP5 up-regulation is accompanied by an increase in the assembly of AKAP5/PKC complexes at the plasma membrane (Figure 2A) [53]. Elevated glucose concentrations also induce diacylglycerol (DAG) synthesis, which, in turn, promotes the chronic activation of anchored PKC [55]. Once activated, PKC promotes the

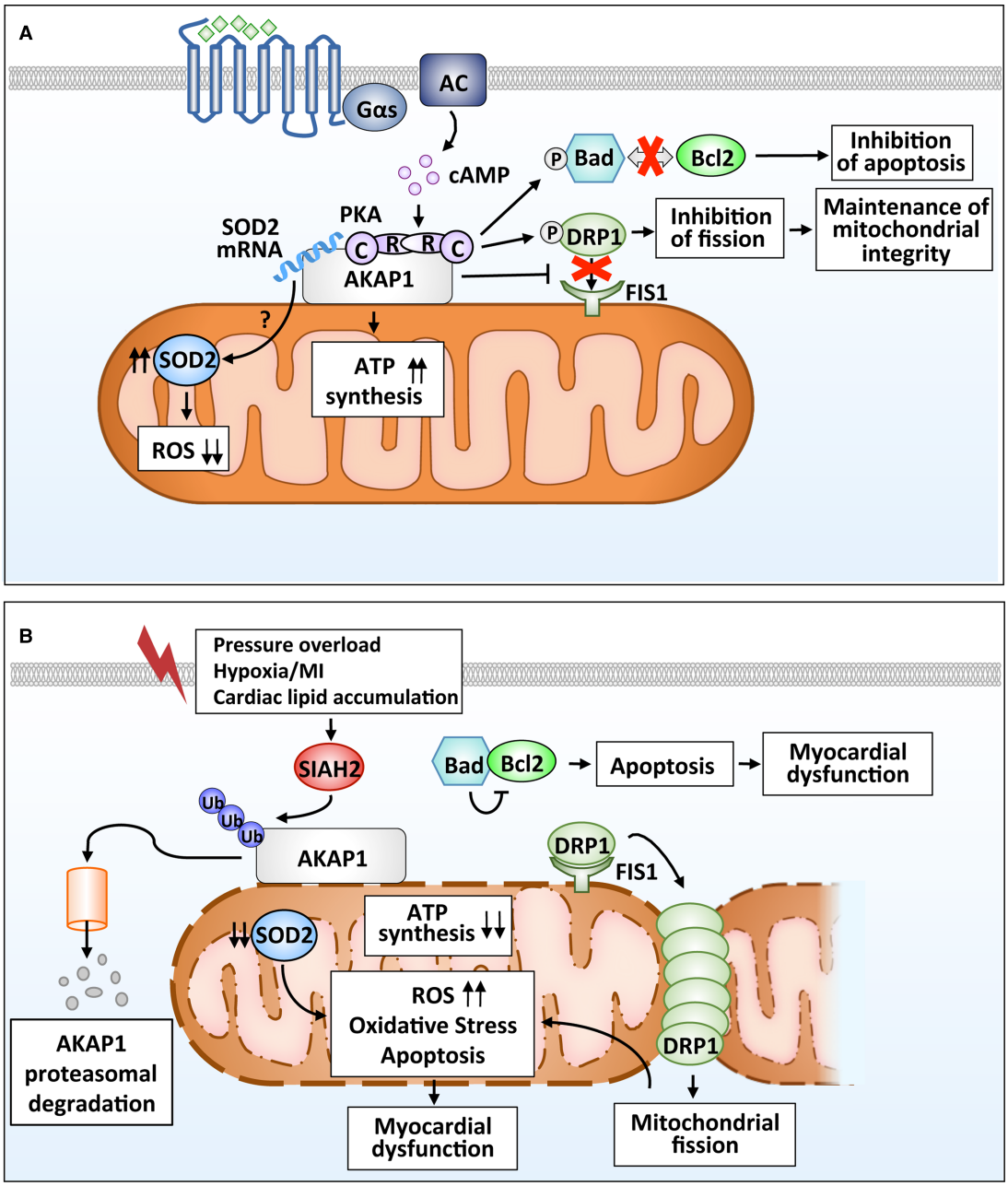

Figure 1. Regulation of mitochondrial integrity, oxidative stress and apoptosis by AKAP1.

(**A**) Under physiological conditions, AKAP1 prevents DRP1 binding with FIS1 by anchoring PKA at the mitochondrial outer membrane. Anchored PKA phosphorylates DRP1 at Ser637, inducing its detachment from the mitochondrial membrane. In addition, AKAP1 physically prevents DRP1/FIS1 interaction, thus inhibiting mitochondrial fission and maintaining mitochondrial integrity. PKA can also phosphorylate Bad at Ser155. As a consequence phosphorylated Bad remains sequestered in the cytoplasm. This prevents it from binding and inactivating Bcl2. Bax-induced apoptosis is therefore inhibited. Despite not having been demonstrated in cardiac cells, AKAP1 has been shown to promote SOD2 mRNA translocation to mitochondria and to the increase mitochondrial SOD2 expression, leading to a reduction in ROS levels. (**B**) Prolonged cardiac stress such as pressure overload, MI-induced hypoxia or myocardial lipid accumulation, induces the activation of SIAH2. SIAH2 promotes AKAP1 ubiquitylation and proteasomal degradation, thus preventing PKA anchoring at the mitochondrial membrane. As a consequence, DRP1 will be able to bind to FIS1 and trigger mitochondrial fission, oxidative stress, and cardiomyocyte apoptosis. In the absence of PKA activity, dephosphorylated Bad forms a dimer with Bcl2, inactivating it and triggering apoptosis. AKAP1 degradation is also correlated with reduced SOD2 levels in mitochondria.

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phosphorylation of $p47^{phox}$, an essential component of the NADPH oxidase, and the p65 subunit of the transcription factor NFκB (Figure 2A) [53]. Phosphorylation of $p47^{phox}$ favors NADPH oxidase activation, ROS production and oxidative stress, whereas phosphorylation of p65 promotes the translocation of NFκB to the nucleus where it induces the transcriptional activation of numerous inflammatory and pro-apoptotic genes (Figure 2A) [53]. Accordingly, AKAP5 knockdown abolishes high glucose-induced $p47^{phox}$ and p65 phosphorylation, oxidative stress, inflammatory responses, apoptosis and diastolic dysfunctions [53]. These findings suggest that coordination of PKC activity by AKAP5 plays a key role in the induction of stress pathways that mediate cardiac glucotoxicity and oxidative stress.

AKAP12/gravin

AKAP12 (or SSeCKS, gravin, AKAP250) was originally characterized as an autoantigen in myasthenia gravis patients [56]. This anchoring protein has been shown to interact with multiple signaling proteins including PKA, PKC, polo-line kinase 1, phosphodiesterases 4D, calmodulin and β-adrenergic receptors (β-ARs) [57,58]. In the cardiovascular system, it is expressed in cardiomyocytes, cardiac fibroblasts and vascular cells. Here, we will exclusively discuss the implication of AKAP12 in cardiac pathophysiology since its role in the vascular system has been reviewed in details previously [16].

Recent evidence indicates that the expression of AKAP12 in mouse hearts is strongly down-regulated in response to exposure to stress molecules such as Ang-II, raising the question of whether a reduction in myocardial AKAP12 levels could contribute to maladaptive remodeling and oxidative stress induced by this agonist [59]. This hypothesis was initially confirmed by studies showing that AKAP12 KO mice subjected to chronic Ang-II infusion experience a more pronounced decline in heart function as compared with wild type mice [59]. Development of this severe phenotype is associated with increased cardiac oxidative stress, inflammation, apoptosis and fibrosis. Investigation of the molecular mechanisms underlying these effects revealed that Ang-II-stimulated AKAP12 KO mice display increased myocardial levels of transforming growth factor β1 (TGFβ1) and sustained phosphorylation and activation of Smad2/3 transcription factors [59]. This suggests that AKAP12 exerts an inhibitory effect on Ang-II induced TGFβ1 expression and signaling (Figure 2B). *In vitro* experiments performed using HL-1 cardiac muscle cells confirmed these findings and further revealed that AKAP12 negatively regulates TGFβ1-induced oxidative stress and transcriptional activation of inflammatory, apoptotic and pro-fibrotic genes (Figure 2B) [59].

The cardioprotective role of AKAP12 is confirmed by additional findings suggesting that the anchoring protein inhibits the detrimental effects of aldosterone on human cardiac fibroblasts [35]. Aldosterone is a mineralocorticoid hormone produced by the adrenal gland and by the stressed or injured myocardium [60]. Accumulating evidence indicates that this hormone can alter cardiac function through its ability to promote mitochondrial dysfunction, oxidative stress and ROS-induced cardiac fibrosis [60].

Chronic stimulation of human cardiac fibroblasts with aldosterone results in a marked down-regulation of AKAP12, which is paralleled by a decrease in the expression of peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), a key regulator of mitochondrial biogenesis, and mitochondrial dysfunction (Figure 2C) [35]. The mechanisms whereby aldosterone controls AKAP12 expression are currently unknown. Importantly, AKAP12 silencing recapitulates the effects of aldosterone since it impairs mitochondrial biogenesis and induces ROS production and oxidative stress [35]. Knowing that oxidative stress is the main inducer of profibrotic responses involved in the differentiation of cardiac fibroblasts to activated myofibroblasts and the overexpression of extracellular matrix proteins, down-regulation of AKAP12 in cardiac fibroblasts is expected to result in myocardial fibrosis and diastolic dysfunctions. This hypothesis will need to be tested *in vivo* using cardiac fibroblast-specific AKAP12 KO mice.

Collectively, these findings suggest that stress signals promote oxidative stress and functional alterations in cardiomyocytes and cardiac fibroblasts, at least in part, through the down-regulation of AKAP12.

AKAP13/AKAP-Lbc

AKAP13 (or AKAP-Lbc) is a multifunctional protein that co-ordinates transduction pathways controlling compensatory and cardioprotective responses in stressed or injured hearts [61]. In the myocardium, its expression is up-regulated in response to multiple stresses and insults including pressure overload, neurohumoral stress [62,63]. It serves as a guanine nucleotide exchange factor (GEF) that controls the activation of the small molecular mass GTPases RhoA and RhoC [64,65], and as scaffolding protein for a multitude of signaling enzymes including PKA, MAPKs, protein kinase Cη (PKCη), protein kinase D1 (PKD1), phosphatases,

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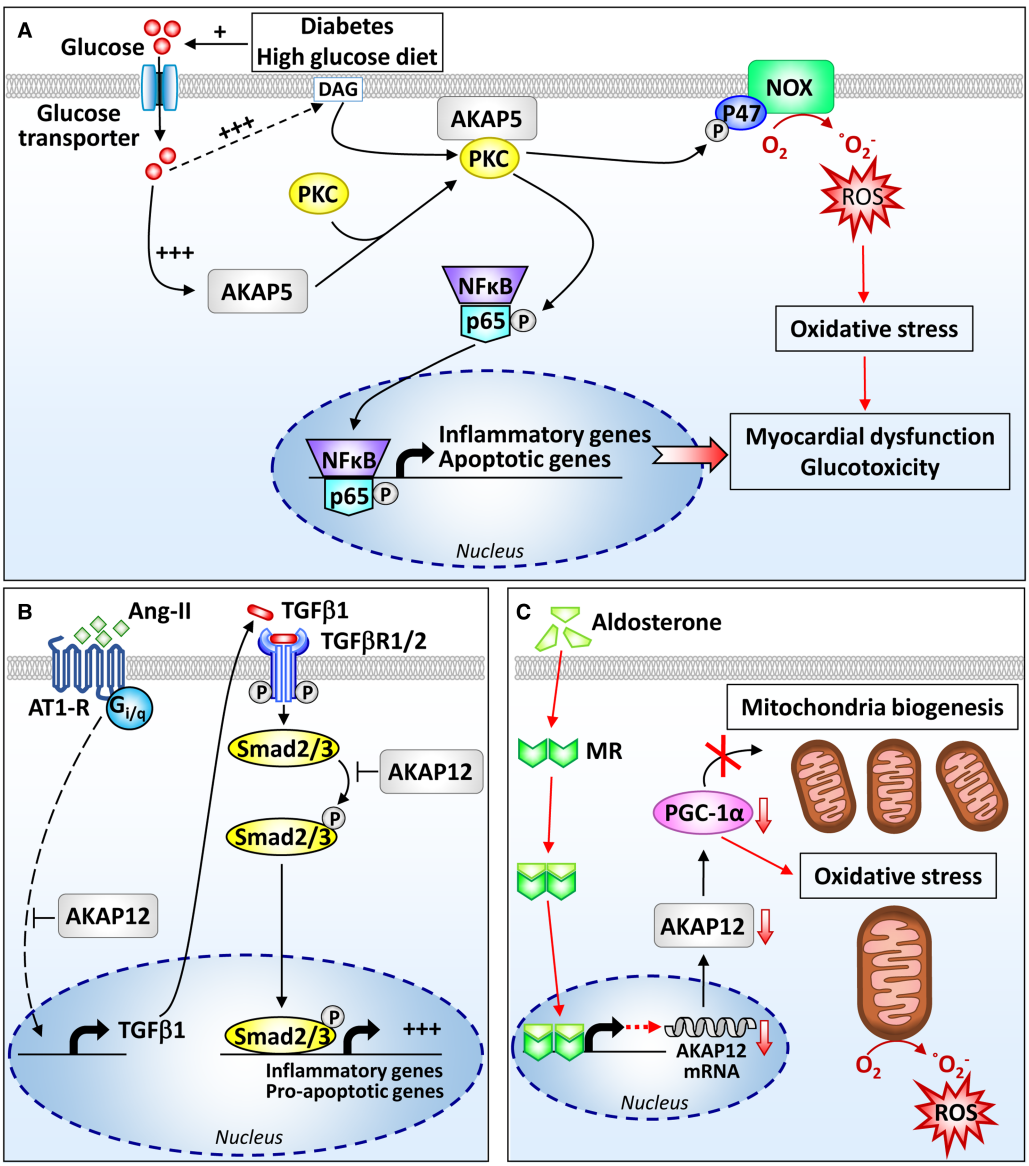

Figure 2. The role of AKAP5 and AKAP12 in modulating oxidative stress in cardiac cells.

(**A**) AKAP5 mediates cardiac glucotoxicity. Elevated extracellular glucose induces the up-regulation of AKAP5 and favors DAG synthesis. This increases plasma membrane anchoring and activation of PKC, which, in turn, phosphorylates $p47^{phox}$ and the p65 subunit of NFκB. $p47^{phox}$ phosphorylation leads to the assembly of Nox at the plasma membrane, ROS production, oxidative stress and cardiomyocyte damage. P65-NFκB phosphorylation leads to NFκB in nuclear translocation, and the consequent transcriptional activation of inflammatory and pro-apoptotic genes, which further enhance myocardial dysfunction. (**B**) AKAP12-mediated inhibition of Ang-II and TGFβ1 signaling in cardiac cells. Ang-II, a ligand that is locally produced by the myocardium in stressed hearts, activates type I Ang-II receptors, which promote TGFβ1 gene activation. Increased TGFβ1 levels, favor the activation of the TGFβ receptor pathway, which leads to the phosphorylation and nuclear translocation of the Smad2/3 complex, and the consequent increase in inflammatory and pro-apoptotic gene expression. AKAP12 reduces inflammatory and apoptotic responses in cardiac muscle cells by exerting an inhibitory effect on Ang-II induced TGFβ1 expression, and TGFβ1-induced Smad2/3 activation. (**C**) The role of AKAP12 in aldosterone-induced oxidative stress in cardiac fibroblasts. Aldosterone, via its mineralocorticoid receptor, decreases AKAP12 mRNA and protein expression. AKAP12 down-regulation is associated with a decrease in PGC-1α, which impairs mitochondrial biogenesis and induces ROS production leading to oxidative stress.

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phosphodiesterases and other signaling proteins [61]. Among the kinases anchored by AKAP13, PKD1 has recently attracted attention because of its implication in prosurvival signaling pathways involved in protecting cardiomyocytes against oxidative stress. In particular, activation of PKD1 by cardioprotective ligands including α1-adrenergic receptor (α1-AR) and sphingosine-1-phosphate receptor (S1P-R) agonists has been shown to

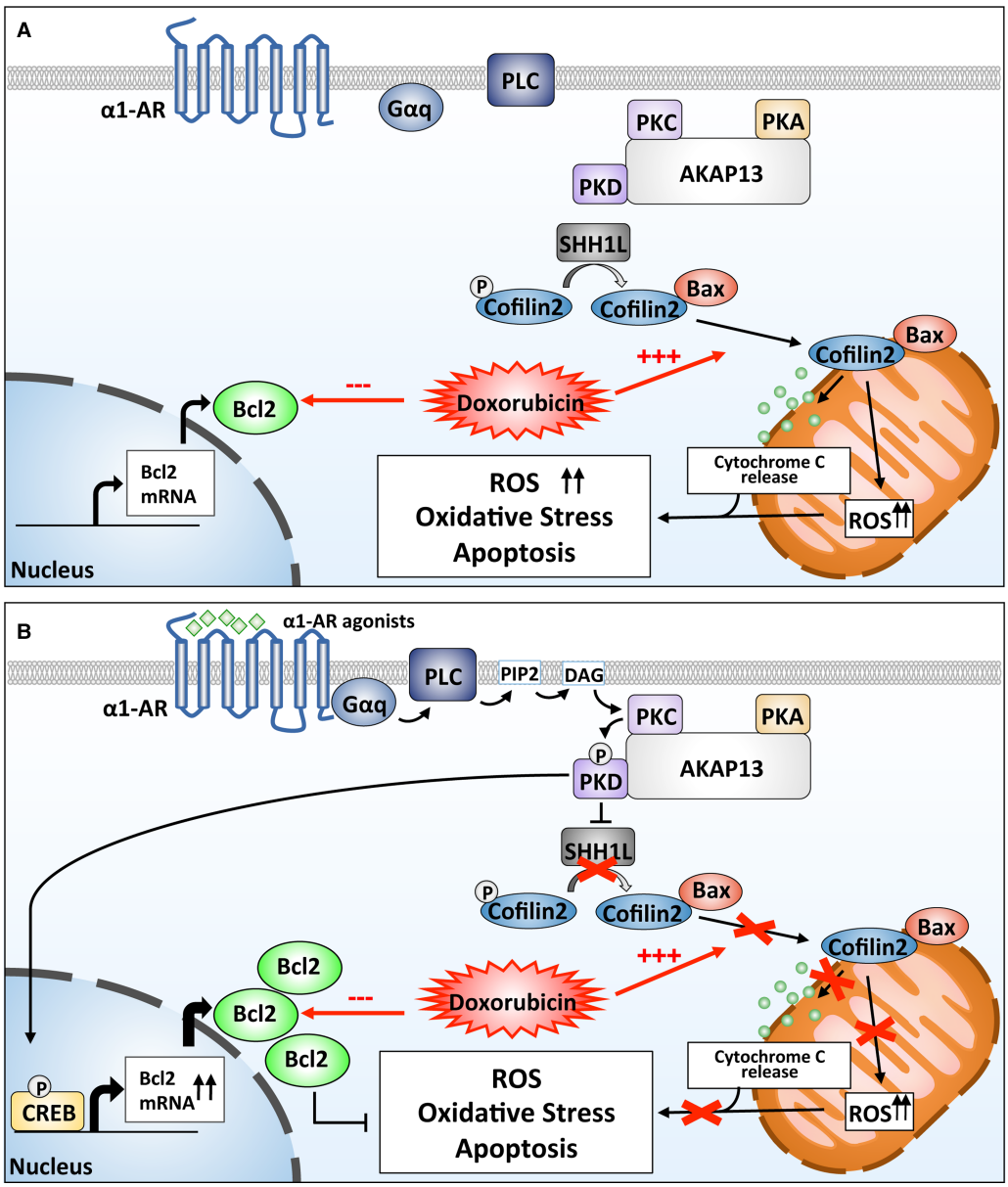

Figure 3. The AKAP13/PKD1 signaling complex protects against Dox-induced cardiomyocyte dysfunction.
(**A**) Dox induces the translocation of cofilin2/Bax complexes to the mitochondria. This leads to mitochondrial dysfunction, ROS production and apoptosis via the release of cytochrome c and subsequent caspase activation. Dox also negatively regulates the transcription of the anti-apoptotic gene Bcl2, which will favor apoptosis. (**B**) AKAP13-anchored PKD1 mediates α1-AR-induced phosphorylation and inactivation of SHH1L. As a consequence cofilin2 dephosphorylation does not occur, which favors the accumulation of phosphorylated cofilin2 in the cytosol. This blocks the ability of Dox to induce the translocation of pro-apoptotic cofilin2/Bax complexes to the mitochondria, thus preventing mitochondrial dysfunction, ROS production and apoptosis. PKD1 also mediates the activation of the transcription factor CREB, which, in turn, promotes the up-regulation of the anti-apoptotic gene Bcl2.

significantly reduce the deleterious effects of ROS in cardiomyocytes subjected to ischemia/reperfusion and the anti-cancer drug doxorubicin (Dox) [66,67].

Dox is an anthracycline widely used to treat a multitude of solid and hematological cancers [3,68]. Despite its efficacy, it can promote severe cardiac side effects, which are the consequence of its ability to accumulate in mitochondria and promote ROS formation, mitochondrial dysfunction, alteration of gene expression and cardiomyocyte apoptosis. Anthracycline-induced cardiac toxicity is a major concern for cancer patients, since it reduces cardiac mass and function and can lead to heart failure [3,68].

Recent evidence now indicates that AKAP13-based signalosomes containing PKD1 mediates the protective effects α1-AR agonists against Dox-induced cardiomyocyte toxicity [66]. Cardioprotection requires the activation of at least two PKD1-dependent pathways that limit the pro-apoptotic effects of Dox-induced ROS formation (Figure 3). On the one hand, activated PKD1 promotes the phosphorylation and inactivation of the slingshot 1L (SSH1L), a phosphatase that selectively dephosphorylate cofilin 2 (Figure 3). This leads to an increase in cofilin2 phosphorylation, which, in turn, inhibits Dox/ROS-induced shuttling of pro-apoptotic cofilin2/Bax complexes from the cytosol to mitochondria, mitochondrial cytochrome C release and caspase 3-induced cardiomyocyte apoptosis (Figure 3) [66]. On the other hand, PKD1 can phosphorylate and activate the cAMP regulatory element-binding protein (CREB), which induces the transcriptional activation of anti-apoptotic genes such as Bcl2 (Figure 2) [66]. Collectively, these findings suggest that the α1-AR/AKAP13/PKD1 pathway confers cardiomyocyte protection against the toxic effects of Dox and that the selective stimulation of α1-ARs could represent a potentially efficient approach to prevent Dox-induced mitochondrial dysfunction, oxidative stress and cardiomyocyte death.

Perspectives

- Redox signaling plays an important role in regulating cardiac function under physiological conditions. However, excessive myocardial production of oxidizing molecules, such a ROS, can lead to local oxidative stress, extensive damage and heart failure. During the last decade, research has been focusing on defining key molecular players involved in stress-induced ROS generation and identifying cardioprotective pathways reducing the extent of ROS-mediated myocardial damage.

- In recent years, AKAPs have been shown to play a crucial role in orchestrating multiple signaling pathways involved in pathological redox signaling in cardiac cells. In particular, AKAPs such as AKAP5 acts as a facilitator of cardiac oxidative stress [53], whereas others including AKAP1, AKAP12 and AKAP13, co-ordinate the activity of PKA and other transduction enzymes to promote cardioprotective signals that inhibit mitochondrial fission, ROS production, oxidative stress and cardiomyocyte apoptosis [35,36,46-48,59,66]. Importantly, it is now demonstrated that stress signals promote cardiac dysfunction by down-regulating the expression of cardioprotective anchoring proteins either by inhibiting their transcription or by increasing their proteolysis. One could, therefore, predict that enhancing the expression and activation of cardioprotective AKAP signalosomes and inhibiting the assembly of AKAP complexes transducing pathological signals in stressed hearts could limit oxidative stress and myocardial damage.

- In this context, based on the data presented above suggesting that α1-ARs activate AKAP13-dependent protective pathways that inhibit Dox-induced mitochondrial damage and oxidative stress in cardiomyocytes one could raise the hypothesis that agonists that selectively activate α1-ARs might offer a therapeutic advantage in preventing Dox-induced cardiac toxicity [66]. In line with this view, it has been recently shown that dabuzalgron, a selective agonist of α1A-AR originally developed to treat urinary incontinence, can efficiently protect mice from the cardiac side effects of Dox treatment, without elevating blood pressure or causing cardiac hypertrophy [69]. Future clinical trials will need to assess whether dabuzalgron induces cardioprotection also in humans and whether these effects require the activation of AKAP13. Based on experimental evidence suggesting that the AKAP5/PKC signaling

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complex acts as a mediator of cardiac oxidative stress and glucotoxicity [53] one could infer that disrupting the interaction of AKAP5 and PKC would inhibit hyperglycemia-induced PKC activation and the consequent induction of pathways promoting ROS formation and inflammation. The development of selective inhibitors of the AKAP5/PKC interaction will require the characterization of the structural determinants involved in the binding of PKC to AKAP5. The identified binding surface could be then targeted by high-throughput virtual screening of compound libraries to identify small molecule inhibitors that could be tested for their ability to reduce hyperglycemia-induced cardiac toxicity. Alternatively, AKAP5–PKC interaction inhibitors could also be directly identified on the basis of functional screenings assessing disruption of AKAP5/PKC complexes. These screening approaches have already been used successfully to identify inhibitors of protein–protein interactions in AKAP signaling complexes [65,70]. In conclusion, based on the experimental evidence accumulated so far, PKA and AKAPs emerge as important regulators of pathological pathways that promote ROS production in the heart. The optimization of experimental approaches enhancing signaling through cardioprotective AKAPs and inhibiting the activity of pathological AKAP transduction complexes might lead to the development of new strategies limiting the impact of oxidative stress on cardiac tissues.

Abbreviations

AKAPs, A-kinase anchoring proteins; Ang-II, Angiotensin II; ASK-1, apoptosis signaling kinase-1; Bad, Bcl2-associated agonist of cell death; Bax, Bcl2-associated X protein; cAMP, cyclic adenosine monophosphate; CREB, cAMP regulatory element-binding protein; CTGF, connective tissue growth factor; D/D, dimerization/docking; DAG, diacylglycerol; Dox, drug doxorubicin; DRP, 1dynamin-related protein 1; EF, ejection fraction; ERKs, extracellular signal-regulated kinases; FIS1, fission protein 1; GEF, guanine nucleotide exchange factor; GLP1, glucagon-like peptide 1; I/R, ischemia/reperfusion; KI, knock-in; KO, knockout; MAPK, mitogen-activated protein kinases; mPTC, mitochondrial permeability transition complex; NADPH, nicotinamide adenine dinucleotide; NF-κB, nuclear factor-κB; NF-κB, nuclear factor Kappa B; NO, nitric oxide; OPA1, optic atrophy 1; PGC-1α, proliferator-activated receptor γ co-activator 1α; PKA, protein kinase; PKCη, protein kinase Cη; PKD1, protein kinase D1; ROS, reactive oxygen species; ROS, reactive oxygen species; RyRs, ryanodine receptors; S1P-R, sphingosine-1-phosphate receptor; SERCA2, sarcoplasmic reticulum calcium ATPase; SIAH2, seven in absentia homolog 2; Sirt3, sirtuin3; SOD2, superoxide dismutase 2; SSH1L, slingshot 1L; TAC, thoracic aortic constriction; TGFβ1, transforming growth factor β1; α1-AR, α1-adrenergic receptor.

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Author Contributions

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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