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Original article

The C-terminus of the long AKAP13 isoform (AKAP-Lbc) is critical for development of compensatory cardiac hypertrophy

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ABSTRACT

The objective of this study was to determine the role of A-Kinase Anchoring Protein (AKAP)-Lbc in the development of heart failure, by investigating AKAP-Lbc-protein kinase D1 (PKD1) signaling *in vivo* in cardiac hypertrophy.

Using a gene-trap mouse expressing a truncated version of AKAP-Lbc (due to disruption of the endogenous AKAP-Lbc gene), that abolishes PKD1 interaction with AKAP-Lbc (AKAP-Lbc-ΔPKD), we studied two mouse models of pathological hypertrophy: i) angiotensin (AT-II) and phenylephrine (PE) infusion and ii) transverse aortic constriction (TAC)-induced pressure overload.

Our results indicate that AKAP-Lbc-ΔPKD mice exhibit an accelerated progression to cardiac dysfunction in response to AT-II/PE treatment and TAC. AKAP-Lbc-ΔPKD mice display attenuated compensatory cardiac hypertrophy, increased collagen deposition and apoptosis, compared to wild-type (WT) control littermates. Mechanistically, reduced levels of PKD1 activation are observed in AKAP-Lbc-ΔPKD mice compared to WT mice, resulting in diminished phosphorylation of histone deacetylase 5 (HDAC5) and decreased hypertrophic gene expression. This is consistent with a reduced compensatory hypertrophy phenotype leading to progression of heart failure in AKAP-Lbc-ΔPKD mice. Overall, our data demonstrates a critical *in vivo* role for AKAP-Lbc-PKD1 signaling in the development of compensatory hypertrophy to enhance cardiac performance in response to TAC-induced pressure overload and neurohumoral stimulation by AT-II/PE treatment.

1. Introduction

Localized regulation and integration of intracellular signal transduction is important for cardiac function. Disruption of appropriate signaling results in the development of heart failure [1,2]. A-Kinase Anchoring Proteins (AKAPs) are fundamental regulatory molecules involved in signal transduction, functioning to target unique signaling complexes to distinct subcellular locations, thereby coordinating signaling and generating substrate specificity [3]. In the heart, AKAPs play a crucial role by integrating cAMP-dependent protein kinase (protein kinase A; PKA) signaling with additional enzymes to modulate physiological functions, including Ca^{2+}-cycling and cardiac contractility [4], as well as pathological processes involved in cardiac remodeling and the development of heart failure [5]. Subcellular localization of signaling components by AKAPs is important for cardiac function. Multiple AKAPs have been identified in cardiac myocytes, targeting signaling complexes to distinct subcellular regions including the sarcolemma [6], sarcoplasic reticulum [7], nuclear envelope [8], and sarcomere [9–11].

A recent proteomic study suggests that differential expression of AKAPs coupled with alterations in the AKAP "interactome" may be critical factors in heart failure [12], however currently, few AKAP knockout or transgenic mouse models have been studied to specifically determine *in vivo* (patho)physiological roles in healthy and diseased heart. Here, we focus on the *in vivo* role of the *AKAP13* gene long transcript, called AKAP-Lbc; due to an N-terminal A-Kinase Anchoring domain [13] and a C-terminal region originally identified in a screen

Abbreviations: 1 M, one month; AT-II, angiotensin II; E/A ratio, early (E) to late (atrial A) ventricular filling velocity ratio; EDT, transmitral early filling deceleration time; EDV, end-diastolic volume; EF, ejection fraction; ET1, endothelin1; FS, fractional shortening; GPCR, G protein coupled receptor; HDAC5, histone deacetylase 5; HOP, hydroxyproline; HW/BW, heart weight to body weight ratio; LV, left ventricular/ventricle; LVAWD, left ventricular diastolic anterior wall thickness; LVPWD, left ventricular diastolic posterior wall thickness; PE, phenylephrine; PKA, protein kinase A; PKC, protein kinase C; PKD, protein kinase D; TAC, transverse aortic constriction; WT, wild type.

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for transforming genes from human myeloid leukemia patients in Lymphoid Blast Crisis [14]. AKAP-Lbc serves as a scaffold for multiple protein kinases, including PKA, protein kinase C (PKCα and PKCη isoforms) and protein kinase D (PKD1) [15]. AKAP-Lbc also acts as a guanine exchange factor (GEF) for Rho [13] and mediates activation of p38α MAPK [16], ERK1/2 [17] and IκB kinase β (IKKβ) [18]. Additionally, we have recently demonstrated that AKAP-Lbc tethers the tyrosine phosphatase Shp2; which is inhibited by PKA phosphorylation in the AKAP-Lbc complex under hypertrophic conditions in the heart [19]. AKAP-Lbc is predominantly expressed in the heart [13] and is essential for cardiac function. Knockout of AKAP-Lbc in mice leads to embryonic lethality due to decreased expression of cardiac developmental genes and deficient sarcomere formation in developing myocytes, resulting in a thin myocardium in the developing heart [20]. Previously, we and others have demonstrated a role for AKAP-Lbc in the induction of cardiac hypertrophy *in vitro* [21,22]. Cardiac myocytes primarily respond to increased workload by an increase in size (hypertrophy). Initially cardiac hypertrophy is a beneficial, compensatory process, decreasing wall stress and increasing cardiac output and stroke volume. However, prolonged hypertrophy is maladaptive, transitioning to decompensation and cardiac failure [23,24]. Understanding how molecular events are orchestrated by AKAP-Lbc may lead to the identification of new pharmacological approaches for the treatment of heart failure.

AKAP-Lbc expression is upregulated in hypertrophic neonatal rat ventricular myocytes (NRVM), whereas siRNA-silencing of AKAP-Lbc expression reduces phenylephrine (PE)-stimulated expression of hypertrophic markers and hypertrophy [21,22]. A similar increase in AKAP-Lbc expression was also observed in human heart specimens obtained from patients with hypertrophic cardiomyopathy where AKAP-Lbc mRNA content was increased, compared to control age-matched healthy human heart samples [21].

In knockdown/rescue experiments using NRVM, to dissect signaling through AKAP-Lbc, our results show that AKAP-Lbc scaffolding of PKD1 is the predominant mechanism of AKAP-Lbc-mediated hypertrophy [21]. Mechanistically, AKAP-Lbc facilitates activation of PKD1 (the predominant protein kinase D cardiac isoform [25–27]) in response to hypertrophic stimuli including PE and endothelin-1 (ET-1). AKAP-Lbc contributes to PKD1 activation in two ways: first, by bringing PKC and PKD1 into close proximity, thereby facilitating phosphorylation and activation of PKD1 by PKC. Second, PKA phosphorylation of AKAP-Lbc, in the PKD1 binding region of AKAP-Lbc (at S2737) releases newly activated PKD1 from the AKAP-Lbc complex. Thus, AKAP-Lbc-anchored PKC and PKA synergistically activate PKD1 by promoting activation and passage of multiple PKD1 molecules through AKAP-Lbc [14].

Activation of PKD1 through AKAP-Lbc facilitates phosphorylation and subsequent nuclear export of histone deacetylase 5 (HDAC5) [21], leading to de-repression of the transcription factor MEF2, resulting in cardiac myocyte hypertrophy through MEF2-mediated transcription of muscle-specific genes and re-expression of developmental genes [28,29]. Currently, the *in vivo* role of this signaling pathway is unknown. Therefore, we set out to determine the role of AKAP-Lbc-PKD1 in the context of pathological hypertrophy and the development of heart failure. In this report, we utilize a gene-trap mouse expressing a form of AKAP-Lbc that is truncated at the C-terminus and unable to bind PKD1. AKAP-Lbc-ΔPKD mice are viable, displaying normal cardiac structure and electrocardiograms. AKAP-Lbc-PKD1 signaling does not appear to be critical for development, but may play a minor role under conditions of β-adrenergic (predominantly Gs-Protein-Coupled Receptor)-induced cardiac hypertrophic remodeling. In response to isoproterenol treatment, mice lacking both the GEF and PKD-binding domains of AKAP-Lbc display abnormal cardiac contractility despite a similar increase in heart size, compared to control wild-type (WT) mice [30].

Here, we demonstrate an *in vivo* role for AKAP-Lbc in the induction of compensatory myocardial hypertrophy in response to pressure overload and angiotensin-II/phenylephrine (AT-II/PE) treatment, both known to activate PKD1, predominantly *via* Gq-PCR mediated pathways.

2. Materials and methods

2.1. Generation of the AKAP-Lbc-ΔPKD mouse

The AKAP-Lbc-ΔPKD mouse was generated from MMRRC gene-trap ES cell line CSJ288 (strain genetic background: B6NCr.129P2) on a C57Bl/6 background and is fully described in [30]. The gene-trap construct uses a strong splice acceptor to create a fused mRNA of upstream AKAP-Lbc exons with the trapping cassette [31]. The AKAP-Lbc-ΔPKD truncation mutant results from specific integration of a β-Geo cassette (β-Galactosidase/neomycin resistance gene) within the endogenous AKAP-Lbc genomic locus, therefore the truncated AKAP-Lbc-β-Geo fusion protein is expressed from the endogenous upstream AKAP-Lbc gene promoter. RT-PCR and sequencing from ES cells and homozygous gene-trap mice confirmed specific gene-trap targeting within the AKAP-Lbc gene.

2.2. Antibodies

Anti-phospho-HDAC4 (Ser246)/HDAC5 (Ser259)/HDAC7 (Ser155) (D27B5) (#3443), anti-HDAC5 (#2082) and anti-PKD1/PKCμ (#2052) were from Cell Signaling Technology, Inc. Anti-cTnI (ab19615) was from AbCam.

2.3. In vitro protein kinase assay

Immune complexes were washed five times with IP buffer (10 mM sodium phosphate, pH 6.95, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1% Triton X-100) and then resuspended in kinase assay buffer (50 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$). Assays were performed as described in [32]. PKD activity assays were carried out using a total reaction volume of 50 μl including 100 μM syntide-2, 5 μM ATP, 5 μCi of [γ-^{32}P]-ATP in kinase assay buffer. Reactions were for 20 min at 30 °C, started upon addition of ATP. Reactions were terminated by centrifugation followed by spotting the reaction mix (40 μl) onto P81 phosphocellulose paper (Whatman). Phosphocellulose papers were washed three times with 75 mM phosphoric acid, once with acetone and then dried. Kinase activity was determined by liquid scintillation counting. PKA activity assays were performed as described for PKD. Prior to assay, PKA catalytic subunit was eluted from AKAP-Lbc immune complexes by adding 50 μl of 10 mM cAMP and incubating for 20 min. PKA assays were carried out in a total reaction volume of 50 μl, using 20 μl of eluted PKA catalytic subunit, 200 μM Kemptide, 5 μM ATP, 5 μCi of [γ-^{32}P]-ATP in kinase assay buffer.

2.4. In vitro Rho-guanine exchange factor (GEF) assay

Following immunoprecipitation of AKAP-Lbc, immune complexes were washed five times with IP buffer and incubated with RhoA-GDP (40 pmol) in binding buffer (50 mM Tris–HCl, pH 7.5, 1 mM DTT, 0.5 mM EDTA, 50 mM NaCl, 5 mM $MgCl_2$, 0.05% polyoxyethylene-10-lauryl ether ($C_{12}E_{10}$), and 10 μM GTPγS with ~500 cpm/pmol [^{35}S]-GTPγS) in a final reaction volume of 50 μl. Reactions were terminated after 20 min incubation at 30 °C by addition of wash buffer. GTPγS binding to RhoA was determined as previously described [33]. [^{35}S]-GTPγS (specific activity = 1250 Ci/mmol) was obtained from PerkinElmer Life Sciences.

2.5. Systemic blood pressure measurement

Intraventricular hemodynamic measurements were carried out using a Scisense FY097B pressure–volume hemodynamics system. Mice were intubated with a 20G angiocath sleeve, connected to a mouse ventilator supplying 1.5% isoflurane mixed with 100% oxygen (Vent settings: Resp rate ≈ 150 bpm and Tidal volume ≈ 15 μl) and then placed supine on a heated pad (37.5 °C). A pressure catheter was