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AKAP-Lbc mediates protection against doxorubicin-induced cardiomyocyte toxicity

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ABSTRACT

Doxorubicin (DOX) is a chemotherapic agent that is widely used to treat hematological and solid tumors. Despite its efficacy, DOX displays significant cardiac toxicity associated with cardiomyocytes death and heart failure. Cardiac toxicity is mainly associated with the ability of DOX to alter mitochondrial function. The current lack of treatments to efficiently prevent DOX cardiotoxicity underscores the need of new therapeutic approaches. Our current findings show that stimulation of cardiomyocytes with the α1-adrenergic receptor (AR) agonist phenylephrine (PE) significantly inhibits the apoptotic effect of DOX. Importantly, our results indicate that AKAP-Lbc is critical for transducing protective signals downstream of α1-ARs. In particular, we could show that suppression of AKAP-Lbc expression by infecting primary cultures of ventricular myocytes with lentiviruses encoding AKAP-Lbc specific short hairpin (sh) RNAs strongly impairs the ability of PE to reduce DOX-induced apoptosis. AKAP-Lbc-mediated cardiomyocyte protection requires the activation of anchored protein kinase D1 (PKD1)-dependent prosurvival pathways that promote the expression of the anti-apoptotic protein Bcl2 and inhibit the translocation of the pro-apoptotic protein Bax to mitochondria. In conclusion, AKAP-Lbc emerges as a coordinator of signals that protect cardiomyocytes against the toxic effects of DOX.

1. Introduction

The increased effectiveness of chemotherapies has reduced cancer–related mortality. However, it has become apparent that chemotherapy can cause severe cardiac co-morbidities that can reduce life quality and affect long-term survival. In this respect, anthracyclines, and in particular Doxorubicin (DOX), are highly efficient chemotherapic agents that are used to treat a variety of hematological and solid tumors despite their well-documented cardiac toxicity [1]. Clinical signs of DOX-induced cardiotoxicity can appear during the first year of therapy and are generally associated with cardiomyocyte death and dilated cardiomyopathy with reduced ejection fraction [2,3].

DOX-induced heart disease has been associated with oxidative stress, alterations of gene expression and iron homeostasis, aberrant regulation of pro- and anti-apoptotic pathways and impaired mitochondrial function and energy production [4–8].

Accumulating evidence indicates that cardiac α1-adrenergic receptors (α1-ARs) produce important pro-survival effects [9–11] and protect cardiomyocytes from DOX-induced cardiac toxicity [12,13]. In particular, the α1-AR-specific agonists phenylephrine (PE) and Dabuzalgron have been shown to efficiently reduce cardiomyocyte apoptosis, interstitial fibrosis and myocardial dysfunction caused by DOX treatment [12,13]. This protective effect relies, at least in part, on the ability of PE to induce the expression of anti-apoptotic proteins of the Bcl2 family [12], and to preserve mitochondrial function [13]. However, a clear understanding on how protective cardioprotective signaling pathways are activated by α1-ARs and how they are coordinated in cardiomyocytes to promote efficient protection against DOX-induced toxicity is still missing.

Scaffolding and anchoring proteins play a central role in coordinating intracellular transduction events in space and time [14]. In particular, A-kinase anchoring proteins (AKAPs) have been shown to orchestrate and synchronize the activity of multiple signaling enzymes to regulate key cellular processes in the heart [15,16]. AKAP-Lbc is an anchoring protein mainly expressed in cardiac tissues, where it functions as a signaling scaffold that coordinates the activation of hypertrophic transduction pathways downstream of α1-ARs [17–19]. *In vivo* experiments provide evidence that AKAP-Lbc promotes compensatory cardiac hypertrophy and cardiomyocyte protection in hearts subjected to pressure overload [20–22]. At molecular level, AKAP-Lbc forms a complex with protein kinase D1 (PKD1) and its upstream activator PKCη, and facilitates activation of PKD1 in response to various

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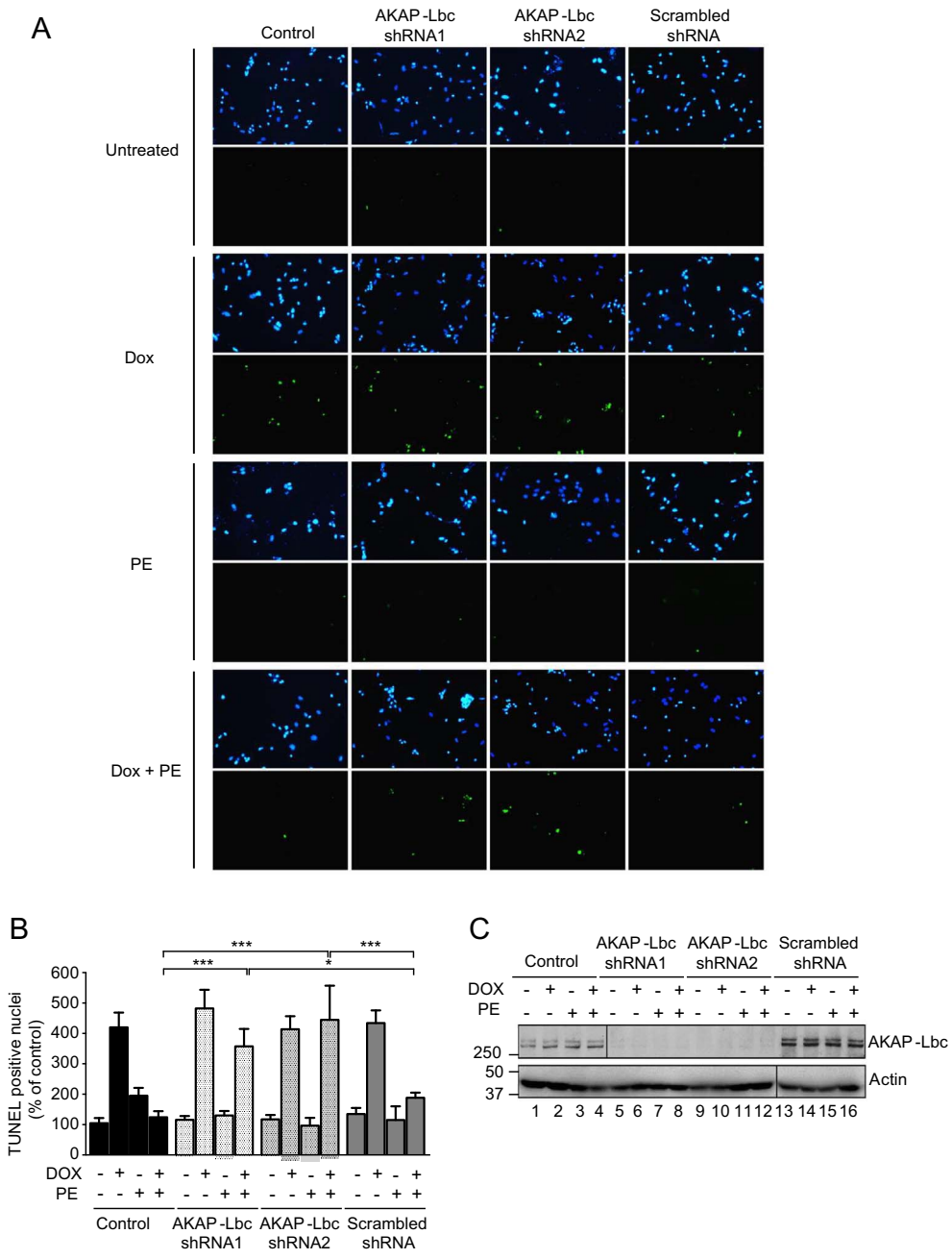

Fig. 1. The α1-AR/AKAP-Lbc signaling axis mediates cardiomyocyte protection against doxorubicin-induced apoptosis. Rat NVMs were infected with control lentiviruses or lentiviruses encoding wild type or scrambled AKAP-Lbc shRNAs at an MOI of 50. 72 h after infection cells were treated for 24 h with 300 nM DOX in the absence or presence of 100 nM PE. A) Representative TUNEL and DAPI staining of cardiomyocytes. Scale bar, 200 μm. B) Quantitation of apoptosis. The number of TUNEL-positive nuclei was normalized to the total number of nuclei per field. TUNEL positive nuclei were counted in 10 fields for each group. C) Silencing of AKAP-Lbc in rat NVMs was confirmed by Western blot using anti-AKAP-Lbc specific antibodies. Actin was used as loading control. Results are expressed as a mean ± SEM of 5 independent experiments. * $p < 0.05$; *** $p < 0.001$.

hypertrophic stimuli [19,23]. Importantly, PKD1 has been shown to regulate the expression of hypertrophic genes as well as to modulate protective responses that maintain mitochondria integrity and function in stressed cardiomyocytes [19,22,24].

In the present study, we provide evidence that AKAP-Lbc acts as a molecular platform that coordinates protective signals preventing DOX-induced cardiomyocyte toxicity. In particular, we could show that stimulation of α1-ARs results in the activation of AKAP-Lbc-anchored PKD1, which, in turn, engages two anti-apoptotic pathways enhancing the expression of Bcl2 and inhibiting the mitochondrial translocation of the pro-apoptotic protein Bax in DOX-treated cardiomyocytes, respectively. These findings indicate that the AKAP-Lbc/PKD1 complex functions to prevent mitochondrial dysfunction and cardiomyocyte death induced by DOX.

2. Material and methods

2.1. Expression constructs

pAB286.1 lentiviral transfer vectors encoding double stranded hairpin (sh) oligonucleotides based upon rat AKAP-Lbc mRNA sequences (GI:198386327, bases 6347–6365 or 6626–6644) were previously described [17]. To generate a lentiviral transfer vector encoding PKD1 shRNA, a fragment containing the sequence encoding for the shRNA was PCR-amplified from the PRKD1-pLKO.1 plasmid (Sigma), and subcloned into the BamHI and SalI sites in the pAB286.1 vector. The lentiviral packaging vectors pCMVΔR8.91 and pMD2.VSVG were described previously [17].

The Flag- and GFP-tagged AKAP-Lbc mutants missing the PKD1 binding domain (AKAP-Lbc-ΔPKD) were generated by deleting the region encoding amino acids 2704–2817 by standard PCR-directed mutagenesis using the Flag-AKAP-Lbc vector [18] and the GFP-AKAP-Lbc