5 Epac1 deletion prevents cardiomyocyte apoptosis during ischemia/reperfusion
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Introduction: Early coronary reperfusion has been established as the best therapeutic strategy to limit infarct size and improve prognosis. Therefore, elucidating the mechanisms underlying cardiomyocyte death may yield novel therapeutic targets to limit ischemia reperfusion (I/R) injury. I/R is accompanied and influenced by perturbations of the β-adrenergic receptor pathway which acts through cAMP-dependent signaling cascade to modulate cardiac function and remodeling.

Purpose: Although the involvement of the cAMP-binding protein Epac1 in cardiac hypertrophy and arrhythmia has been recently described, its role in I/R-induced cardiomyocyte death has not yet been investigated.

Methods: Isolated adult cardiomyocytes from Epac1 knock-out (Epac1-/-) mice or wild-type (WT) littermates were exposed to hypoxia (HX) for 4h and 2h of reoxygenation period (HX+R). Cell death was determined by Trypan blue staining and LDH release. The mitochondrial permeability transition pore (mPTP) opening was monitored by the calcein loading CoCl2-quenching technique. The area at risk was examined by Evans blue and infarct size was evaluated by TTC staining.

Results: Our data showed that HX+R-induced cardiomyocyte death were significantly prevented in adult cardiomyocytes isolated from Epac1-/- mice. In addition, we found that the increased expression of apoptotic markers (Bax, cleaved Caspase-9, Caspase-3) during HX+R conditions were also inhibited in Epac1-/- cardiomyocytes compared to WT cells. Interestingly, HX+R induced a decrease in calcine fluorescence corresponding to mPTP opening (56% ± 1.1 vs control cells set at 100%) in WT cardiomyocytes while genetic deletion of Epac1 prevented mPTP opening in HX+R conditions. Conversely, we found that the infarct size was significantly reduced in the Epac1-/- mice compared to the WT animals (33 ± 2% vs 33 ± 4%, p<0.001) despite the same area at risk.

Conclusion: Epac1 deletion confers resistance to I/R injury via the inhibition of a mitochondrial death signaling. Our study shed light the therapeutic potential of the inhibition of Epac1 and the development of Epac1 inhibitors as new drugs to treat I/R injury.

6 Subcellular redistribution of mitogen and stress activated kinase 1 (MSK1) contributes to protection against oxidative stress-induced apoptosis in cardiac myocytes
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MSK1 is a predominantly nuclear kinase known to associate with a variety of nuclear targets, including transcription factors and chromatin-associated proteins, in several tissues. However, scarce evidence indicates that this kinase may also have cytoplasmic targets. In the heart, we have previously shown that MSK1 phosphorylates CREB and contributes to the hypertrophic response. In this study, we aimed to determine the subcellular distribution of MSK1 and its role in response to oxidative stress in cardiomyocytes. Exposure of cardiac myocytes to H2O2 or simulated ischemia (S) resulted in increased phosphorylation of MSK1, which is mediated by p38 MAPK and/or ERK1/2. Subcellular localization of endogenous MSK1 was determined under confocal laser scanning microscope using immunofluorescence and antibodies to both total- and phosphoprotein. Upon exposure to oxidative stress, a significant percentage of nuclear MSK1 was translocated to the cytoplasm. In addition, pretreatment of cardiac myocytes with the MSK1 inhibitor SB747651A resulted in increased levels of apoptosis either at basal conditions or after oxidative stress. Furthermore, inhibition of MSK1 downregulated the anti-apoptotic Bcl-2 protein. The results demonstrate that, altered distribution of MSK1 may lead to modulation of cytoplasmic targets, including apoptosis associated proteins, contributing to cardioprotection during oxidative stress.

7 Excessive ROS production in mitochondria switches off protective mitochondrial kinase signaling
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Background: Accumulating evidence indicates that both anti-survival and pro-survival signaling mechanisms are localized within mitochondria, among which glycogen synthase kinase 3 β (GSK-3 β) plays crucial roles in regulation of the mitochondrial permeability transition pore (mPTP). We recently demonstrated that GSK-3 β translocates to the mitochondria in response to oxidative stress, promoting production of excess reactive oxygen species (ROS) and opening of the mPTP. Here, we examined how ROS modify mitochondrial pro-survival kinases.

Methods and Results: Isolated mitochondria from Hek293 cells were incubated in a vehicle or trypsin at 1–1,000 μmol/L. Immunoblot analysis revealed that TOM20, an outer membrane (OM) protein, cytochrome oxidase IV, an inner membrane (IM) protein, and cyclophilin D, a matrix protein, were digested by 1, 100 and 1,000 μmol/L of trypsin, respectively, confirming stepwise digestion of mitochondrial membranes. At baseline, 56%, 13% and 21% of mitochondrial GSK-3 β were localized in OM, IM and matrix, respectively, the other 11% presumably being in the intermembrane space. A similar pattern of intra-mitochondrial localization was observed for ERK. In contrast, only 10% of mitochondrial Akt was observed in OM, and 32% and 26% of Akt were in IM and matrix, respectively. Treatment with IGF-1 (10 nmol/L, 45 min) increased total mitochondrial GSK-3 β by 1.4 fold, with its increase in IM being prominent (2.6 fold), compared to those in vehicle-treated cells. The level of phospho-ERK in OM was increased by 1.5 fold, and a marked increase in IM by 13.5 fold was observed. IGF-1 increased phospho-Akt in OM and IM by 1.8 and 2.9 fold, respectively, though such an increase was not observed for phosphorylation of both kinases in the matrix. Addition of antymycin A (40 μmol/L, 30min), a mitochondrial ROS inducer, to IGF-1 eliminated phosphorylation of Akt, ERK and GSK-3 β in all compartments. AA did not change intra-mitochondrial localization of Akt and ERK but slightly increased total GSK-3 β level.

Conclusion: The present results suggest that Akt and ERK in OM and/or IM contribute to protective Ser9-phosphorylation of GSK-3 β in mitochondria and that excessive mitochondrial ROS production switches off the protective signaling by dephosphorylation of mitochondrial Akt and ERK possibly via activation of phosphatases.

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