Protein Kinase Cδ Activation Induces Apoptosis in Response to Cardiac Ischemia and Reperfusion Damage

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Heart attacks caused by occlusion of coronary arteries are often treated by mechanical or enzymatic removal of the occlusion and reperfusion of the ischemic heart. It is now recognized that reperfusion per se contributes to myocardial damage, and there is a great interest in identifying the molecular basis of this damage. We recently showed that inhibiting protein kinase Cδ (PKCδ) protects the heart from ischemia and reperfusion-induced damage. Here, we demonstrate that PKCδ activity and mitochondrial translocation at the onset of reperfusion mediates apoptosis by facilitating the accumulation and dephosphorylation of the pro-apoptotic BAD (Bcl-2-associated death promoter), dephosphorylation of Akt, cytochrome c release, PARP (poly(ADP-ribose) polymerase) cleavage, and DNA laddering. Our data suggest that PKCδ activation has a critical pro-apoptotic role in cardiac responses following ischemia and reperfusion.

Reperfusion after coronary occlusion is one of the leading causes of cardiac injury and cell death (1). A variety of signaling pathways participate in myocardial responses to ischemia and reperfusion and the damage that occurs involves both necrotic and apoptotic cell death (2, 3). Apoptosis, otherwise known as programmed cell death, is an energy-dependent process (4) that requires the participation of specific cascades of tightly regulated enzymes that function to remove injured, dying, or unnecessary cells (3). Apoptosis can be activated by a number of stimuli such as DNA damage (5) and oxidative stress (6–8). Unlike apoptosis, necrosis is an energy-independent process that often occurs in response to uncontrolled cell rupturing, leading to death of surrounding cells and a subsequent inflammatory response (9).

Current research of the molecular events that control the apoptotic process in response to ischemia and reperfusion implicated pathways involving death receptor-induced apoptosis by, for example, up-regulation of their ligands such as Fas (10–12) and tumor necrosis factor-α (13). Other works described the effects of differential expression and activity of the Bcl-2-related proteins, such as Bak, BAD, Bax, Bid, Bcl-2, and Bcl-xL (14–18). In addition, signaling kinases including p38 MAPK (19), JNK (6), Erk (20), Akt (21), and protein kinase C (PKC) (22) (reviewed in Ref. 3) have also been suggested to play a role in apoptosis. These enzymes and others cooperate to induce downstream events resulting in cytochrome c release, caspase activation, PARP cleavage, and DNA laddering (22–24). However, how these receptors, kinases, and other enzymes influence apoptosis caused by ischemia and reperfusion injury is still under investigation.

One family of proteins that has been shown to mediate ischemia and reperfusion damage is PKC. Since PKC was first identified in 1977 (25), PKC activity has been shown to affect numerous signal transduction processes including differentiation, tumor progression, proliferation, secretion, as well as apoptosis (3, 26). A hallmark of PKC activation involves the movement, or translocation, of PKC from the cytosol to membranes in response to various stimuli (27). In isolated adult cardiac myocytes, ischemia induces the activation and translocation of two PKC isozymes, PKCe and δ (28, 29). However, using PKC isozyme-specific peptides that modulate individual isozyme translocation and activity (29), we showed that PKCe and δ activities have opposing consequences in response to ischemia-induced cell damage (30, 31). Activating PKCe during ischemia leads to cardioprotection (29, 32, 33), whereas activating PKCδ with a PKCδ-specific activator enhanced cell damage (29). Recent evidence indicates that PKCδ, but not PKCe, also participates in reperfusion injury. Delivery of the PKCδ-selective inhibitor peptide, ωV1-1, to hearts ex vivo or in vivo (30, 31) during the onset of reperfusion was cardioprotective. In contrast, delivery of ωRACK peptide to activate PKCe translocation during reperfusion had no cardioprotective effect (30). Our data suggest that activation of PKCδ during reperfusion mediates cardiac damage. However, questions remain regarding the molecular basis of the damage that occurs to the heart in response to PKCδ activity.

Many studies suggest a role for PKCδ in apoptosis (reviewed in Ref. 34). In both LNCAP prostate cancer cells and CaCo-2 human colon-cancer cells, phorbol 12-myristate 13-acetate-induced translocation of overexpressed PKCδ results in apoptosis (35, 36). In addition, PKCδ-induced apoptosis occurs in response both to UV as well as etoposide-induced damage (37, 38). PKCδ translocates to the mitochondria, resulting in the release of cytochrome c from the mitochondria into the cytosol, leading to the activation of the caspase cascade and, ultimately, the cleavage of PARP. The cleaved PARP is then cleaved again into a series of smaller fragments, resulting in the characteristic DNA laddering pattern.

The abbreviations used are: PKC, protein kinase C; MAPK, mitogen-activated protein kinase; JNK, c-Jun NH2-terminal kinase; Erk, extracellular signal-regulated kinase; PARP, poly(ADP-ribose) polymerase; MOPS, 4-morpholinepropanesulfonic acid; PBS, phosphate-buffered saline; TUNEL, transferase-mediated dUTP nick end labeling.

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release of cytochrome c, in response to various pro-apoptotic stimuli (39–41). Therefore, the pro-apoptotic activity of PKCδ may mediate mitochondrial dysfunction. In this study, we provide evidence that PKCδ translocation to the mitochondria during reperfusion plays a key role in the regulation of the mitochondrial involvement in the signaling pathways leading to apoptosis and cell death.

**Experimental Procedures**

**Isolation and Rat Heart Perfusion**

Male Wistar rats (250–300 g) were heparinized (2000 units/kg intraperitoneally) and then anesthetized with sodium pentobarbital (100 mg/kg intraperitoneally). The hearts were rapidly excised and then perfused with an oxygenated (95% O2, 5% CO2) Krebs-Henseleit buffer containing (in mmol/liter) NaCl 120, KCl 5.8, NaHCO3 25, NaH2PO4 1.2, MgSO4 1.2, CaCl2 1.0, and dextrose 10, pH 7.4, at 37 °C in a Langendorff coronary perfusion system as previously described (30).

The hearts were perfused with a constant coronary flow rate of 10 ml/min throughout the experiment. After a 10-min equilibration, perfusion was stopped for 30 min to induce global ischemia, during which time the hearts were immersed into a 37 °C heated bath of Krebs-Henseleit buffer. After the ischemic period, the flow was restored; and reperfusion commenced for 15, 30, 60, or 120 min with or without 500 nm 8V1-1 (29), conjugated to the cellular permeable, Tat (amino acids 47–57, Tat-8V1-1 for short) (42), for the first 15 min of reperfusion. In situ, Figure 5B, reperfusion was studied for up to 240 min. Following reperfusion, the hearts were removed from the apparatus and homogenized for mitochondria isolation, sliced into sections, or snap-frozen with liquid nitrogen, stored at −70 °C, and subsequently used for protein analysis. All animal protocols were approved by the Institutional Animal Care and Use Committee of Stanford University and the Case Western Reserve University.

**Isolation of Subsarcolemmal Mitochondria from Rat Heart**

Immediately following each experimental protocol, the hearts were immersed and rinsed in ice-cold homogenization buffer A (210 mm mannitol, 70 mm sucrose, 1.0 mm EDTA, and 5.0 mm MOPS at pH 7.4). Hearts (0.9–1.2 g) were minced and homogenized in 20 ml of homogenization buffer A using a polytron homogenizer (low setting, 2 s, three times) as previously described (43, 44). The homogenate was filtered through cheesecloth and centrifuged at 10,000 × g for 10 min (5 °C) to obtain the mitochondrial pellet and the cytosolic extract (supernatant). The mitochondrial pellet was rinsed and resuspended in buffer A at a final concentration of ~20 mg/ml. Protein determinations were carried out using the bicinchoninic acid method (Pierce) with bovine serum albumin as a standard. Protein determinations were carried out using the bicinchoninic acid method (Pierce) with bovine serum albumin as a standard.

**Western Blot Analysis**

**Detection of PKCδ and Cytochrome c—PKCδ levels were assessed using 30 μg of mitochondrial protein/lane resolved by 4–15% SDS-PAGE and transferred to nitrocellulose. Proteins from cytosolic extracts were resolved by 4–20% SDS-PAGE and transferred to nitrocellulose. After transfer, the membranes were washed three times in phosphate-buffered saline with 0.05% Tween 20 (PBST). The membranes were then blocked for 30 min in PBST with 5.0% milk and probed with polyclonal anti-PKCδ (Cell Signaling Technology), cytochrome c, adenine nucleotide translocase (obtained from Dr. Schmidt, Hormel Institute), and glyceraldehyde-3-phosphate dehydrogenase (Chemicon International) antibodies, followed by secondary anti-IgG antibody bound, and a 505–550-nm band pass filter for the green. Image analysis was performed using Meta Morph software (Universal Imaging).

**TUNEL Staining**—To observe the incidence of apoptosis in damaged tissue, we subjected isolated perfused rat hearts to 30-min of ischemia and then 60 min of reperfusion. Tissue sections were immediately fixed in 4% formaldehyde and dried for 15 min at room temperature, and the coverslips (Corning) were mounted using Vectashield (Vector Laboratories). Fluorescence images of mitochondria (MitoTracker) and PKCδ (fluorescein isothiocyanate) were obtained using a laser scanning confocal microscope (Pascal, Zeiss) with a 63× water objective (1.2 numerical aperture). The slides were imaged on the stage of an inverted microscope (Axiovert 100) using the 568-nm laser line for the red label and the 488-nm line for green. Emission was collected through a 585-nm-long pass filter for the red label, and a 505–550-nm band pass filter for the green. Image analysis was performed using Metamorph software (Universal Imaging).

**DNA Laddering**—To observe the appearance of DNA laddering, ~100 mg of ex vivo rat heart tissue following ischemia and reperfusion was isolated, and the protein concentrations were determined by Bradford assay. For PKCδ translocation, ~5 μg of soluble and particulate fractions were resolved by 10% SDS-PAGE and transferred to nitrocellulose membrane. After transfer, the blots were blocked with a 5% milk solution in Tris-buffered saline (pH 7.5) containing 0.05% Tween 20 (TBST) and probed with polyclonal anti-PKCδ (Cell Signaling Technology) and anti-PKCδ (Santa Cruz Biotechnology) antibodies followed by secondary anti-IgG rabbit antibody linked to horseradish peroxidase (Amersham Biosciences). Protein bands were visualized using ECL and quantitated using NIH ImageJ software.
We reported that inhibiting PKCδ activity during reperfusion after ischemia, using Tat-δV1-1 (δV1-1), improved myocardial cell survival both \textit{ex vivo} as well as \textit{in vivo} (30, 31). To identify the molecular events affected by inhibiting PKCδ activation during ischemia and reperfusion, we first confirmed previous observations (28, 29) that PKCδ translocates from the cell soluble to the cell particulate fraction in response to ischemia (Fig. 1B, \textit{I}_{30} versus N). However, we observed that significant increases in PKCδ translocation occur during reperfusion (Fig. 1B, \textit{I}_{30}/R_{60}).

**RESULTS**

\textbf{δV1-1 Inhibits PKCδ Translocation during Reperfusion—}We observed that significant increases in PKCδ translocation occur during reperfusion (Fig. 1B, \textit{I}_{30}/R_{60}). Delivering 500 nm of δV1-1 at the onset of reperfusion resulted in a significant decrease in PKCδ translocation, whereas delivering the Tat carrier alone had no effect (data not shown). The δV1-1 effect was specific for PKCδ, because treatment with δV1-1 did not affect aPKC (Fig. 1C). Therefore, reperfusion-induced PKCδ translocation in this rat Langendorff preparation is inhibited with δV1-1 administration during the initiation of reperfusion.

\textbf{δV1-1 Inhibits Reperfusion-induced PKCδ Translocation to Mitochondria and Cytosol Translocation—}In cultured myeloid leukemia, muscle, and liver cells, PKCδ was shown to translocate to mitochondria upon insulin, H$_2$O$_2$, or 12-O-tetradecanoylphorbol-13-acetate treatment (40, 41, 46). We therefore determined whether our observed increased translocation of PKCδ to the particulate fraction is due, at least in part, to its translocation to the mitochondria upon insulin, H$_2$O$_2$, or 12-O-tetradecanoylphorbol-13-acetate treatment (40, 41, 46). We therefore determined whether our observed increased translocation of PKCδ to the particulate fraction is due, at least in part, to its translocation to the mitochondria upon insulin, H$_2$O$_2$, or 12-O-tetradecanoylphorbol-13-acetate treatment (40, 41, 46). We therefore determined whether our observed increased translocation of PKCδ to the particulate fraction is due, at least in part, to its translocation to the mitochondria upon insulin, H$_2$O$_2$, or 12-O-tetradecanoylphorbol-13-acetate treatment (40, 41, 46).
were treated during the first 15 min of reperfusion with δV1-1, no reperfusion-induced increase in mitochondrial PKCδ levels was observed. In addition, the Tat carrier peptide alone did not inhibit PKCδ translocation to the mitochondria (not shown). The purity of the mitochondrial fraction and equal protein loading were confirmed by probing both mitochondrial and
cytosolic fractions for the mitochondrial protein adenine nucleotide translocase and the cytosolic marker glyceraldehyde-3-phosphate dehydrogenase. Therefore, PKCδ translocation to the mitochondria during reperfusion appears to be specific.

To further establish whether PKCδ and mitochondria co-localized in tissue, we labeled fixed heart tissue sections from each condition with the mitochondrial-selective dye MitoTracker CMXRos (red) and a fluorescein isothiocyanate-conjugated secondary antibody against primary anti-PKCδ (green) (Fig. 3). Using confocal laser fluorescence microscopy, we observed a dramatic increase in the distribution of co-localized PKCδ and mitochondria (yellow) in hearts reperfused after global ischemia (Fig. 3B), whereas very little co-localization was evident in normoxic (Fig. 3A) and ischemic hearts (Fig. 3B). Importantly, and correlating with our fractionation stud-

**Fig. 3.** Reperfusion-induces co-localization of PKCδ and mitochondria. Representative confocal images of heart sections from previously described treated and nontreated groups are shown. The right column is a merge of MitoTracker (red, left column) and PKCδ (green, middle column). Co-localized PKCδ and mitochondria represented by yellow staining (C, right column). The images represent three independent and double-blinded experiments from four random sections/treatment.

**Fig. 4.** δV1-1 treatment inhibits caspase 3 activation and PARP inactivation. Protein (30 μg/lane) from whole cell lysates from hearts treated as previously described were resolved on 10–12.5% SDS-PAGE and caspase 3 (A) and PARP (B) contents were determined. The blots are representative of four to five independent experiments, and the quantitated data (means ± S.D.) relative to perfused normoxic values (100%) are shown in the lower panels; n = 4. *, p ≤ 0.05 in A and 0.02 in B.

**Fig. 5.** TUNEL staining and DNA laddering of δV1-1-treated and nontreated reperfused hearts. A, hearts that underwent 30 min of ischemia followed by 60 min of reperfusion were sliced and probed for the appearance of DNA fragmentation using the TUNEL method. Sixty minutes of reperfusion induced significant TUNEL-stained nuclei of cardiac cells (white arrows) as compared with the normoxic control hearts. Treatment with δV1-1 greatly inhibited the appearance of TUNEL-positive nuclei. Tri-color staining was performed to detect cardiac myocytes. Staining is as follows: blue, α-actinin; green, 4,6-diamidino-2-phenylindole; yellow, TUNEL. TUNEL-positive nuclei were counted in a total of 1,500 myocytes over several random fields and expressed as percentages of the total number of nuclei. The values represent the means ± S.D. *, p ≤ 0.05 versus N; **, p ≤ 0.003 versus I/R. B, representative photograph of two independent experiments of DNA extracted from normoxic (N), ischemic (I30), and reperfused (I30/R60) 500 nM Tat-δV1-1) hearts electrophoresed on a 1.8% agarose gel.
ies studies (Fig. 2), when we inhibited PKCδ translocation at the beginning of reperfusion using δV1-1, the co-localization of PKCδ with mitochondria was greatly inhibited (Fig. 3D).

Western blot analysis also revealed a significant increase in the cytosolic content of cytochrome c during reperfusion (Fig. 2B), concurrent with PKCδ translocation to mitochondria (Fig. 2A). This increase in cytosolic cytochrome c was blocked by the addition of δV1-1 at the onset of reperfusion (Fig. 2B), whereas the Tat carrier alone had no effect (not shown). Probably because of mechanic disruption of the mitochondria during isolation, a significant level of cytochrome c is present in cytosolic extracts prepared from perfused normoxic control hearts (Fig. 2B). Nevertheless, the increase during reperfusion appears specific, given that it is dependent on PKCδ translocation, and the mitochondrial protein adenine nucleotide translocase does not appear in the cytosolic fraction under any of the experimental conditions (Fig. 2C).

δPKC Inhibition during Reperfusion Decreases Caspase 3 and PARP Cleavage and DNA Fragmentation—Release of cytochrome c from the mitochondria into the cytosol induces caspase activation involved in apoptosis (reviewed in Ref. 47). We found a significant increase in caspase 3 cleavage and activation, as shown by a decrease in procaspase 3, in response to reperfusion (Fig. 4A). Furthermore, reperfusion led to the increase of another apoptotic marker, cleaved PARP, as shown by a decrease in full-length PARP in response to reperfusion (Fig. 4B). PARP becomes inactivated upon cleavage and can no longer participate in DNA repair or maintain genomic stability (48). Administration of δV1-1 during reperfusion inhibited caspase 3 activation and PARP inactivation (Fig. 4).

To confirm that ischemia and reperfusion induced apoptosis, we examined tissue sections of hearts for cleaved DNA using the TUNEL assay (49). Indeed, reperfusion following ischemia induced a significant increase in TUNEL-stained nuclei; treating hearts with δV1-1 caused a greater than 70% reduction in TUNEL staining (Fig. 5A). We confirmed that reperfusion induced apoptosis by measuring DNA fragmentation (45). We observed significant DNA laddering in hearts exposed to 4 h of reperfusion following 30 min of global ischemia (Fig 5B). Treating hearts with δV1-1 to inhibit reperfusion-induced PKCδ translocation led to a marked inhibition of DNA ladder formation (Fig. 5B). Therefore, our findings support previous data suggesting that ischemia and reperfusion induces myocardial cell apoptosis (31, 50, 51) that is mediated by PKCδ translocation to the mitochondria and the activation of the apoptotic effectors involving cytochrome c release, caspase 3 activation, PARP cleavage, and DNA fragmentation.
PKCδ Activation during Reperfusion Affects BAD Protein Levels, BAD Phosphorylation, and Akt Activity—To further investigate the role of PKCδ in myocardial cell apoptosis, we examined the levels and activity of the Bcl-2-related proteins: a pro-apoptotic protein, BAD, as well as anti-apoptotic proteins Bcl-2, Bcl-xL, and Akt. Ischemia and reperfusion caused significant increases in the levels of BAD protein (Fig. 6A), as well as considerable reductions in Bad phosphorylation (Fig. 6B) and the levels of anti-apoptotic Bcl-2 and Bcl-xL (Fig. 6, D and E). These results suggest that ischemia and reperfusion induced changes in the ratios and activity of pro- and anti-apoptotic proteins. When we specifically inhibited PKCδ activity by treating hearts with δV1-1 during reperfusion, only, the levels of BAD were reduced, whereas BAD phosphorylation increased to the levels present during basal perfused conditions, with no changes in the ischemia and reperfusion-induced decreases in Bcl-2 or Bcl-xL. Therefore, our results suggest that increased PKCδ translocation correlates with a concurrent increase in BAD protein and decrease in phosphorylated BAD, but changes in Bcl-2 and Bcl-xL are independent of PKCδ activity.

The phosphatidylinositol 3-kinase-related kinase Akt plays a part in the survival pathways of many different cell types (52, 53) by phosphorylating BAD, which enables its binding to cytosolic 14-3-3 and keeps it away from the mitochondria (54, 55). In the heart, active phosphorylated Akt has been shown to protect cardiac cells from ischemia and reperfusion damage and apoptosis (21, 56). We therefore determined whether ischemia and reperfusion caused changes in Akt activity and whether PKCδ inhibition would have any Akt-specific effects. There was a significant decrease in active Akt, as shown by a decrease in phosphorylated Akt during reperfusion (Fig. 6C) (no changes in fold levels of Akt occurred). Importantly, we found that inhibiting PKCδ translocation during reperfusion blocked the decrease in Akt phosphorylation (Fig. 6C), which may lead to inhibition of BAD phosphorylation. Therefore, these data suggest that PKCδ activity may mediate ischemia and reperfusion-induced apoptosis by specifically decreasing the activity and phosphorylation of pro-survival Akt and pro-apoptotic BAD.

DISCUSSION

Our results show that ischemia and reperfusion cause translocation of PKCδ to mitochondria, which in turn affects the activity of downstream apoptotic factors through the release of cytochrome c. In addition, reperfusion induced significant increases in the levels of pro-apoptotic BAD and decreases in BAD and Akt phosphorylation, as well as anti-apoptotic Bcl-2 and Bcl-xL protein levels. Using δV1-1 to specifically inhibit PKCδ translocation at the onset of reperfusion, we observed inhibition of cytochrome c release, caspase 3 activation, PARP cleavage, and apoptosis-related DNA fragmentation. In addition, inhibiting PKCδ translocation at reperfusion attenuated the rise in the levels of pro-apoptotic BAD and led to increased BAD phosphorylation and the activity of the pro-survival kinase Akt. However, inhibiting PKCδ had no effect on the decline in the levels of the anti-apoptotic proteins Bcl-2 and Bcl-xL. Therefore, our data demonstrates that PKCδ increases ischemia and reperfusion-induced apoptosis by affecting the balance between the pro-apoptotic and the anti-apoptotic enzymes.

Cell survival and death relies on the balance of pro- and anti-apoptotic proteins (57). Increases in BAD protein levels with parallel decreases in anti-apoptotic Bcl-2 in isolated porcine lung endothelial cells was shown to be sufficient for apoptosis induction (58). Moreover, Schimmer et al. (59) observed that overexpression of BAD causes apoptosis in Cos cells, whereas in cardiac myocytes, H₂O₂ induces an increase in BAD protein levels that lead to apoptosis as well (60). Increasing the amount of anti-apoptotic Bcl-2 through overexpression in cardiac myocytes has also been shown to inhibit apoptosis caused by ischemia and reperfusion both in vitro and in vivo (61, 62). In our study, we observed changes in the phosphorylation state of BAD, an event that correlates with BAD inactivation because of its phosphorylation by Akt and sequestration by 14-3-3 (54, 55, 63). Previous studies support our finding that changes in the ratios and phosphorylation states of pro- and anti-apoptotic proteins are sufficient in determining cell fate (59–62). How PKCδ activation regulates the phosphorylation state of BAD and Akt has not yet been determined. It is also not clear whether PKCδ is the Akt and/or BAD kinase. As for regulation of BAD levels by PKCδ, it is possible that the inhibition of proteosome function that we previously reported (64) and other unknown signaling enzymes are contributors.

In summary, we show that reperfusion causes translocation of PKCδ to mitochondria where it enhances the release of cytochrome c, leading to the propagation of further downstream apoptotic effects (Fig. 7). More importantly, the selective PKCδ inhibitor, δV1-1, not only attenuates translocation of PKCδ to the mitochondria but also prevented the cascade of events that lead to apoptosis; inhibiting PKCδ lead to a decrease in BAD protein levels as well as an increase in BAD and Akt phosphorylation. Coupled with the ischemia and reperfusion-induced decreased Bcl-2 and Bcl-xL, the increase in Akt and BAD phosphorylation along with the decrease in BAD levels may shift cells toward a survival state. Lowering BAD levels and increasing phosphorylated BAD may block the release of cytochrome c, possibly preventing Bax homodimerization (65, 66). Once cytochrome c release is blocked, caspase 3 activation that induces PARP cleavage and DNA fragmentation are also inhibited (Fig. 7). Therefore, blocking PKCδ translocation appears to be an effective means of salvaging the reperfused myocardium after an ischemic insult.

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REFERENCES

1. Yellon, D. M., and Baxter, G. F. (2000) Heart 93, 381–387
2. Anversa, P., Cheng, W., Liu, Y., Leri, A., Redaelli, G., and Kajstura, J. (1998) Basic Res. Cardiol. 93, 8–12
3. Clerk, A., Cole, S. M., Cullingford, T. E., Harrison, J. G., Jormakka, M., and Valka, D. M. (2003) Pharmacol. Ther. 97, 223–261
4. Bujia, L. M. (1998) Lab. Invest. 78, 1345–1373
5. Rich, T., Allen, R. L., and Wyllie, A. H. (2000) Nature 407, 777–783
6. Dougherty, C. J., Kubasik, L. A., Prentice, H., Andreka, P., Bishopric, N. H., and Webster, K. A. (2002) Biochem. J. 362, 561–571
7. Strasser, A., O’Connor, L., and Dixit, V. M. (2000) Annu. Rev. Biochem. 69, 217–245
8. Yamamoto, S., Seto, K., Morisco, C., Vattner, S. F., and Sadoshima, J. (2001) J. Mol. Cell Cardiol. 33, 1829–1848
9. Hawkins, H. K., Entman, M. L., Zhu, J. Y., Youker, K. A., Berens, K., Dare, M., and Smith, C. W. (1996) Circ. Res. 78, 784, H456–H463
10. Takemura, G., Kato, S., Aoyama, T., Hayakawa, Y., Kanoh, M., Maruyama, R., Araki, M., Nishigaki, K., Minagouchi, S., Fukuda, K., Fujii, T., and Fujii, H. (2001) J. Biol. Chem. 276, 546–556
11. Jeremias, I., Kupatt, C., Martin-Villaherm, B., Habazettl, H., Schenkel, J., Boekstegers, P., and Debattin, K. M. (2000) Circulation 102, 915–920
12. Lee, P., Sata, M., Lefer, D. J., Factor, S. M., Walsh, K., and Kasis, R. N. (2003) Am. J. Physiol. 284, H456–H463
13. Paz, Y., Frolik, I., Pevni, D., Shapira, I., Yuhas, Y., Iaina, A., Wellman, Y., Chernichovsky, T., Nesher, N., locker, C., Mohr, R., and Uretzky, G. (2003) J. Am. Coll. Cardiol. 42, 1295–1305
14. Chen, M., He, H., Zhan, S., Krajewski, S., Reed, J. C., and Gottlieb, A. R. (2001) J. Biol. Chem. 276, 30724–30728
15. Murriel, C., and Mochly-Rosen, D. (2003) Arch. Biochem. Biophys. 420, 246–254
16. Imahashi, K., Schneider, M. D., Steenbergen, C., and Murphy, E. (2004) Circ. Res. 95, 734–741
17. Gustafsson, A. B., Tsai, J. G., Logue, S. E., Crow, M. T., and Gottlieb, R. A.
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(2004) J. Biol. Chem. 279, 21233–21238

18. Suzuki, K., Murtaza, B., Smolenetz, R. T., Sammut, I. A., Suzuki, N., Kaneda, Y., and Yacoub, M. H. (2001) Circulation 104, Suppl. 1, 1303–1313

19. Mackay, K., and Mohly-Rosen, D. (1999) J. Biol. Chem. 274, 6272–6279

22. Freude, B., Masters, T. N., Robicsek, F., Fokin, A., Kostin, S., Krajewski, S., Suzuki, K., Murtuza, B., Smolenski, R. T., Sammut, I. A., Suzuki, N., Kaneda, Y., and Yacoub, M. H. (2001) Circulation 104, Suppl. 1, 1303–1313

21. Fujio, Y., Nguyen, T., Wencker, D., Kitsis, R. N., and Walsh, K. (2000) Circulation 101, 660–667

22. Freude, B., Masters, T. N., Robicsek, F., Fokin, A., Kostin, S., Krajewski, S., Reed, J. C., Olivetti, G., and Anversa, P. (2000) J. Mol. Cell. Cardiol. 32, 197–208

23. de Moissac, D., Gurevich, R. M., Zheng, H., Singal, P. K., and Kirshenbaum, L. A. (2000) J. Mol. Cell. Cardiol. 32, 53–63

24. Heidkamp, M. C., Bayer, A. L., Martin, J. L., and Samarel, A. M. (2001) Circ. Res. 89, 882–890

25. Takai, Y., Kishimoto, A., Inoue, M., and Nishizuka, Y. (1977) J. Biol. Chem. 252, 7693–7699

26. Newton, A. C. (1995) J. Biol. Chem. 270, 28495–28498

27. Kraft, A. S., Anderson, W. B., Cooper, H. L., and Sando, J. J. (1982) J. Biol. Chem. 257, 13193–13196

28. Gray, M. O., Karliner, J. S., and Mohly-Rosen, D. (1997) J. Biol. Chem. 272, 30945–30951

29. Chen, L., Hahn, H., Wu, G., Chen, C. H., Liron, T., Schechtman, D., Cavallaro, G., Baciocchi, E., Wu, G., Hahn, H., Osinska, H., Liron, T., Lorenz, J. N., and Mohly-Rosen, D. (2003) Proc. Natl. Acad. Sci. U. S. A. 98, 11114–11119

30. Inagaki, K., Hahn, H. S., Dorn, G. W., and Mohly-Rosen, D. (2003) Circulation 108, 869–875

31. Inagaki, K., Chen, L., Ikeno, F., Lee, F. H., Imahashi, K., Bouley, D. M., Rezaee, M., Tock, P. G., Murphy, E., and Mohly-Rosen, D. (2003) Circulation 108, 2303–2307

32. Dorn, G. W., II, Sourroujon, M. C., Liron, T., Chen, C. H., Gray, M. O., Zhou, H. Z., Csukai, M., Wu, G., Lorenz, J. N., and Mohly-Rosen, D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12798–12803

33. Mohly-Rosen, D., Wu, G., Hahn, H., Osinska, H., Liron, T., Lorenz, J. N., Yatani, A., Robhins, J., and Dorn, G. W., II (2000) Circ. Res. 86, 1173–1179

34. Brodie, C., and Slagle, J. (2000) Circulation 101, 649–655

35. Cerda, S. R., Bissonnette, M., Scaglione-Sewell, B., Lyons, M. R., Khare, S., Mustafi, R., and Brasitus, T. A. (2001) J. Mol. Cell. Cardiol. 33, 65–75

36. Chen, N., Ma, W., Huang, C., and Dong, Z. (1999) J. Biol. Chem. 274, 15389–15394

37. Blass, M., Krones, I., Kazimirska, G., Bumbar, P. M., and Brodie, C. (2002) Mol. Cell. Biol. 22, 182–195

38. Matassa, A. A., Carpenter, L., Biren, T. J., Muzinich, M. J., and Reyland, M. E. (2001) J. Biol. Chem. 276, 29719–29728

39. Majumder, P. K., Pandey, P., Sun, X., Cheng, K., Datta, R., Saxena, S., Kharbanda, S., and Kufe, D. (2000) Cell Growth & Differ. 12, 465–470

40. Schwartz, S. R., Ho, A., Voero-Akhan, A., and Dowdy, S. F. (1999) Science 285, 1569–1572

41. Lucas, D. T., and Szwedz, L. I. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 510–514

42. Lucas, D. T., and Szwedz, L. I. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6689–6693

43. Pozi, S., Malferrari, G., Biunno, I., and Kazanietz, M. G. (2002) Cell Physiol. Biochem. 12, 39–46

44. Matassa, A. A., Carpenter, L., Biren, T. J., Muzinich, M. J., and Reyland, M. E. (2001) J. Biol. Chem. 276, 29719–29728

45. Pozzi, S., Malferrari, G., Biunno, I., and Kazanietz, M. G. (2002) Cell Physiol. Biochem. 12, 39–46

46. Caruso, M., Muitan, M. A., Bifarcal, G., Miele, C., Vigliotta, G., Oriente, P., Formisano, P., and Breginout, F. (2001) J. Biol. Chem. 276, 45088–45097

47. Gavrieli, Y., Sherman, Y., and Ben-Sasson, S. A. (1992) J. Cell Biol. 119, 493–501

48. Yang, E., Zha, J., Jockel, J., Boise, L. H., Thompson, C. B., and Korsmeyer, S. J. (1996) Cell 80, 285–291
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