Kinase activity-independent anchoring function of protein kinase C-δ. Focus on “Protein kinase C-δ regulates the subcellular localization of Shc in H₂O₂-treated cardiomyocytes”

Yan Zhang,¹ Chun-Mei Cao,¹ and Rui-Ping Xiao¹,²,³

¹Institute of Molecular Medicine, Peking University, Beijing, China; ²Drug Discovery Center, School of Chemical Biology and Biotechnology, Shenzhen Graduate School of Peking University, Shenzhen, China; and ³Laboratory of Cardiovascular Sciences, National Institute on Aging, National Institutes of Health, Baltimore, Maryland

PROTEIN KINASE C (PKC) plays a pivotal role in mediating cellular responses to extracellular stimuli and is involved in various fundamental cellular processes, including cell proliferation, differentiation, apoptosis, hypertrophic growth, and exocytotic release in the cardiovascular system as well as in noncardiovascular cells such as neurons, islet cells, chromaffin cells, and paramecium (3, 16, 20, 22, 25). The PKC family consists of about 10 serine/threonine protein kinases that share certain basic structural features (19). A highly conserved COOH-terminal catalytic domain is required for ATP/substrate-binding and catalysis, whereas an isoenzyme-specific NH₂-terminal regulatory domain contains an autoinhibitory pseudosubstrate domain and two discrete membrane-targeting modules (termed C1 and C2) (19, 25). On the basis of the properties of their NH₂-terminal regulatory domains, the PKC family is further classified into three subfamilies, conventional (cPKC including PKCα, -βI, -βII, and -γ), novel (nPKC including PKCδ, -ε, -η, and -θ), and atypical PKC (aPKC including PKCζ and -λ).

The classic view of the activation of PKC is based on studies of cPKCs such as PKCα, a key event of the signal transduction of G protein-coupled receptors, particularly G₁₁-coupled receptors. Under resting conditions, PKCα localizes to the cytosolic fraction. Agonist stimulation of G₁₁-coupled receptors leads to an increase in two second messengers, Ca²⁺ and diacylglycerol (DAG), which bind to the regulatory domain of PKC, resulting in activation and membrane translocation of the kinase (18). In contrast, aPKCs are not allosterically regulated by Ca²⁺ or DAG, while the activation of nPKCs depends on the presence of DAG but not Ca²⁺ (19, 25).

Unique Regulatory Mechanism Governing PKCδ Activation

There are some unique features of PKCδ regulation and functionality relative to other members of PKC family. Importantly, activation of PKCδ is regulated by phosphorylation of specific tyrosine (Tyr) sites, in addition to the classic DAG-dependent mechanism (24). Mouse, rat, and human PKCδ contains 19, 21, and 20 Tyr residues, respectively. Tyr phosphorylation is a unique regulatory mechanism for PKCδ, compared with other members of the PKC family. Among all the phosphorylation sites, Tyr³¹¹ and Tyr³³² are the most well characterized (4, 12). Previous studies have revealed that phosphorylation of Tyr³¹¹ and Tyr³³² is important for the activation of PKCδ in response to oxidative stress (12, 17). The phosphorylation of these sites occurs by different kinases with Tyr³¹¹ by non-receptor-type kinases such as Src and Lck family (4, 12) and Tyr³³² by Lyn (another Src family kinase) and epidermal growth factor receptor (13, 17). Furthermore, PKCδ can be cleaved by proteolysis to generate a catalytically active fragment at the onset of apoptosis in response to various death-inducing stimuli such as DNA-damaging drugs, ionizing radiation, tumor necrosis factor, and anti-Fas antibody (6, 7, 8).

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15, 26). Caspase 3 and related proteases are responsible for this degradation of PKCδ (26).

In the heart, PKCδ has been implicated in myocardial contraction, ischemic preconditioning (IPC), cardiac hypertrophy, and heart failure (10, 20, 29). PKCδ has been defined as a primary mediator of IPC protection (23, 31), during which transient ischemia protects against subsequent severe ischemia-reperfusion (IR) injury. Among all the members of the PKC family, PKCδ appears to play the most crucial role in IPC protection (5, 11, 14, 32), although PKCε, -α, and -η also participate in IPC response (21, 28, 30). Evidence for cardioprotection by PKCδ includes the markedly exaggerated myocardial IR damage that is observed in PKCδ knockout mice (14), although other studies have shown inconsistent results (5).

Despite the well-established biological and pathological actions of PKCδ, a key question is which action(s) depend on phosphorylation of PKCδ at Tyr sites and which are mediated by the classic DAG-dependent mechanism? The answer to this question will not only further understanding of PKC signaling but also bear important therapeutic implications.

**Kinase Activity-Independent Anchoring Function of PKCδ and Its Potential Pathological Relevance**

It has been controversial as to whether the physical interaction between PKCδ and Shc (Src homologous and collagen) depends on the phosphorylation status of PKCδ. While some previous studies have shown that PKCδ phosphorylation promotes the formation of the PKCδ-Shc complex (13, 17), other studies argue against this idea (13). In the current issue of *American Journal of Physiology-Cell Physiology*, Guo and colleagues (9) demonstrate that, in cultured cardiomyocytes subjected to H2O2 treatment, PKCδ forms a complex with the Shc protein. Although H2O2-induced formation of the PKCδ-Shc complex is accompanied by an elevation of the phosphorylation level of Tyr332 of PKCδ, this complex is insensitive to an inhibitor of Src kinases [4-amino-5-(4-methylphenyl)-7-(t-buty1)pyrazolo[3,4-d]pyrimidine, PP1], which inhibits Tyr phosphorylation of PKCδ, or GF109203X (GFX), which inhibits the catalytic activity of PKCδ. Moreover, H2O2 treatment induces the translocation of PKCδ-Shc complex to a detergent-insoluble fraction and mitochondrial compartment in cardiomyocytes subjected to oxidative stress. Thus, the study by Guo and colleagues clearly demonstrates that neither the phosphorylation nor catalytic activation of PKCδ is required for the H2O2-induced physical interaction of PKCδ with Shc or the subsequent translocation of the complex in neonatal cardiomyocytes (9).

Consistent with the study by Guo and colleagues, studies in rat aortic smooth muscle cells have provided evidence that PKCδ exhibits kinase-independent functions (8).

What is the functional consequence of oxidative stress-induced, kinase activity-independent translocation of PKCδ-Shc complex? Previous studies have shown that the translocation of Shc to a detergent-insoluble fraction (mainly intermediate filament proteins) is crucial for the function of Shc (2, 27) in activating the Ras-Raf-MAPK signaling cascade. Shc translocates to activated receptor and binds to Grb2, which, in turn, interacts with the guanine nucleotide exchange protein Son of Sevenless (SOS), resulting in the translocation of SOS to the plasma membrane, and the activation of the MAPK signaling pathway (1). The study by Guo et al. provides mechanistic insights, in particular, showing that, in response to oxidative stress, PKCδ acts as a scaffold protein that anchors Shc and facilitates the temporal and spatial organization of important signaling molecules, including Shc, Grb2, and SOS1 (Fig. 1) (9). Future studies are needed to determine 1) whether the oxidative stress-induced, kinase activity-independent translocation of PKCδ-Shc leads to the activation of the Ras-MAPK signaling pathway and, if so, 2) whether it contributes to the pathogenesis of cardiac hypertrophy and heart failure.

In summary, the study by Guo et al. has defined a novel kinase activity-independent function of PKCδ in anchoring and targeting Shc to detergent-insoluble fraction and mitochondrial compartment in cardiomyocytes subjected to oxidative stress. This finding sheds new light on our understanding of the signaling and biology of PKCδ. This study may also bear important pathogenic significance, in particular, in myocardial IR injury and other forms of oxidative stress-related cardiovascular diseases.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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